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Dynamics of dissolved organic matter and its bioavailability to heterotrophic bacteria in the Gulf of Finland, northern Baltic Sea

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Dr. Risto Lignell designed the experiments and monitoring programme in I–III (DOM project) and Dr. Anssi Vähätalo designed IV (DOM photochemistry project).

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IV Hoikkala gave the original idea of measuring bacterial community composition and designed this part of the study. Hoikkala implemented the study with MSc. Hanna Aarnos, Dr. Anssi Vähätalo and Dr. Risto Lignell. Hoikkala analysed the bacterial community composition data and wrote the manuscript. All authors contributed with comments.

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Dissolved organic matter (DOM) in surface waters originates from allochthonous and autochthonous sources, the latter of which includes exudation by phytoplankton, viral lysis of planktonic organisms and "sloppy" feeding by zooplankton. The concentration of DOM in seawater exceeds by one to two orders of magnitude that of particulate organic matter. Thus the DOM pool may be crucial to nutrition of pelagic osmotrophs, such as bacteria and algae, which are capable of exploiting dissolved organic substrates. In this thesis, monitoring surveys and laboratory experiments were used to examine the seasonal dynamics of DOM, including interactions of DOM and heterotrophic bacteria, in the Gulf of Finland, northern Baltic Sea, which is rich in allochthonous humic DOM. Despite the large ambient DOM pools and their potentially marked influence in the planktonic food webs and biogeochemical cycles of carbon and nutrients, few investigations in the Baltic Sea have focused on the dynamics of DOM, and information from the Gulf of Finland is almost lacking.

In this thesis, seasonal changes in the net pools of dissolved organic C (DOC), N (DON) and P (DOP) were followed along with ambient key physical, chemical and biological variables on a shore-to-open-sea salinity gradient once in January and biweekly during the phytoplankton growth season. Horizontal coverage of these data was complemented with DOM samplings along a transect from the western to the eastern part of the Gulf. The monitoring study showed that autochthonous DOM accumulates throughout the productive season and that the accumulated DOM is N- and P-rich compared with the bulk DOM pool in the surface layer of the Gulf of Finland. Notable DOM accumulation occurred during the actively growing and declining phases of spring and late summer blooms. Total export estimates of surface DOC, DON and DOP by autumn overturn corresponded to about 11–25 % of reported annual particulate organic matter sedimentation in our study area.

Seasonal variation in the availability of the net DOC and DON pools for bacterial utilization was investigated with incubations of natural bacterial samples for 2–3 weeks. The concentrations of labile DOC were low in spring and during the summer minimum period, whereas the pools of labile DON were more variable. The labile DOM accumulated during and after the late summer cyanobacterial bloom, with low C:N ratios. For determination of factors that control the net DOM pools, limitation of bacterial growth by inorganic nutrients (N and P), labile C and temperature was followed in natural surface and deep-water bacterial samples during the main postspring bloom stages of phytoplankton growth. Agreeing with the low degradability of the ambient DOC pool, bacterial production was consistently C-limited in the surface layer, with N or both N and P as the secondary limiting nutrients from spring to early summer and in late summer, respectively. In deep water, bacterial growth showed combined temperature and C limitation.

Sunlight induces photochemical transformation of DOM, and the importance of this process to bacterial growth during summer was investigated with samples representing extensive spatial and temporal coverage. In addition, photochemical transformation of refractory DOM and its effects on growth and composition of the microbial community were studied in further detail during a late summer cyanobacterial bloom. Photochemical

transformation of DOM generally resulted in increased bacterial production, and photoproduced labile DOC was estimated to support < 10 % of the daily bacterial C production in the surface layers during summer. Photochemical transformation of DOM led to clear changes in the composition of the bacterial community, with notable increases in the relative percentage of a few typical freshwater bacteria. The results further indicated that bacterial taxa benefiting from labile photoproducts included specialists growing strictly on the photoproducts of humic matter.

The results of this thesis suggest that the C-limited bacterial community is for most of the productive season capable of efficient utilization of the labile C compounds released in the Gulf of Finland. The accumulation of phytoplankton-derived, autochthonous DOC during the productive season and subsequent DOC export to deep water are thus lower than in situations where nutrient-limited bacteria would allow accumulation of labile DOC, decreasing the efficiency of the plankton system in incorporating atmospheric CO_2. Nevertheless, accumulation in the DOM pool forms a notable temporary storage of phytoplankton-derived C, N and P. The pool of labile DON, which accounted for up to 95 % of the available N in surface water during summer, is a notable nutrient source for the N-limited plankton community. Photochemical transformation of DOM seems to contribute relatively little to the bacterial C demand, which is satisfied by autochthonous DOM released from the plankton food web in the Gulf of Finland. However, photoproduction of labile DOM appears to have notable qualitative effects on the composition of the bacterial community, probably contributing to the success in the Baltic Sea of bacteria originating in freshwater.

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CONTENTS

1. INTRODUCTION

1.1. Flows of DOM in the marine microbial food webs

The vastness of marine dissolved organic matter (DOM) pools implies their great importance to marine ecosystems and the biogeochemical cycle of C and nutrients. Marine DOM contains a C mass of approx. 700 Gt, which is comparable to that of atmospheric CO_2 (approx. 750 Gt C; Siegenthaler & Sarmiento 1993) and DOM concentrations in seawater exceed by one to two orders of magnitude those of particulate organic matter (POM; e.g. Williams 1995, Zweifel et al. 1995). In addition to C, the DOM pool functions as a notable storage of the macronutrients, N and P. Most of the dissolved N (averaging 60–69 %) in all aquatic environments but marine deep waters is in the dissolved organic N (DON) pool (summarized in Bronk 2002), making DON a potentially important nutrient source, especially in N-limited marine areas, such as most of the Baltic Sea (e.g. Kivi et al. 1993, Lignell et al. 2003). Similarly, the percentage of dissolved organic P (DOP) is large in marine surface waters, ranging from approx. 30–100 % of the total dissolved P (summarized in Karl & Björkman 2002).

Within the vast pool of marine organic matter, the mass of living organisms of approx. 3 Gt C is vanishingly small (Siegenthaler & Sarmiento 1993), but its functions in the food web are central to determining the flows and net pools of DOM and POM. In marine areas, a major part of the DOM pool is ultimately derived from primary production within various food web processes. Notable DOM production occurs via extracellular release by phytoplankton, due to "sloppy" feeding and excretion by grazers, release from bacterioplankton, viral lysis of phytoplankton and bacterioplankton cells and solubilization of particles by bacterial ectoenzymes (e.g. Thingstad et al. 1997, Azam 1998, Nagata 2000, Ward & Bronk 2001, Carlson 2002). In coastal areas, allochthonous inputs from terrestrial sources and from primary production in rivers present other marked sources of DOM. In the Baltic Sea, allochthonous DOM forms approx. 60 % of the total DOM pool (Alling et al. 2008). A large fraction of the nutrients introduced to coastal waters is bound to DOM, the fractions of DON and DOP averaging 41 % and 18 % of the total riverine N and P loads to the Baltic Sea, respectively (Stepanauskas et al. 2002). Allochthonous DOM loads clearly have the potential to affect the level of primary production, thus contributing to algal blooms and the trophic state of the system.

Heterotrophic bacteria are the major consumers of DOM in surface waters, processing about 50 % of the primary production in marine and fresh waters (Ducklow & Carlson 1992). Part of the DOM bound to the bacterial biomass is transferred to higher trophic levels in the "micobial loop", first by heterotrophic nanoflagellates (HNF) grazing on bacteria and further by microzooplankton grazing on HNF, and thus becomes available to the "classical" food web mediated by large zooplankton (Azam et al. 1983). Due to the many trophic steps included, the transfer of C is inefficient in the "microbial loop" compared with that of the short "classical" large algae-zooplankton link, and a large part of the photoassimilated C is lost in respiration. The efficiency of bacteria in binding the utilized dissolved organic C (DOC) to their biomass, i.e. bacterial growth efficiency (BGE), varies with the trophic state of the system, ranging from 0.01 to 0.66, with median values of 0.22 and 0.32 for oceanic and coastal areas,

respectively (reviewed in del Giorgio & Cole 1998).

The availability of the DOM pool for bacterial degradation forms a continuum from most labile compounds, with turnover times from hours to days (Keil & Kirchman 1999, Skoog et al. 1999), to refractory compounds that resist degradation for millennia (Williams & Druffel 1987, Bauer et al. 1992). Most labile compounds, such as dissolved free amino acids and glucose, form important C and N sources for bacteria and are thus found in marine waters only in nanomolar concentrations (Keil & Kirchman 1999, Skoog et al. 1999). Biologically labile DOC (LDOC), degradable within 1–2 weeks, ranges in marine surface waters from negligible to approx. 30 % of the total DOC pool (e.g. Søndergaard & Middelboe 1995, Raymond & Bauer 2000, Carlson et al. 2002, Hopkinson et al. 2002), with an average value for several marine areas of 19 % (Søndergaard & Middelboe 1995).

Investigations in which LDOC, labile DON (LDON) and labile DOP (LDOP) have been simultaneously measured imply that the degradability of DOM components increases in the order DOC < DON < DOP (Hopkinson et al. 2002, Lønborg et al. 2009). In marine waters, LDON and LDOP pools, degradable within weeks, have accounted for 4–29 % and 32–60 % of the respective total DOM pools (Jørgensen et al. 1999, Hopkinson et al. 2002, Nausch & Nausch 2007). Within months, natural bacteria have been able to deplete on average 40–74 % and 82–88 % of the DON and DOP pools, respectively (Hopkinson et al. 2002, Lønborg et al. 2009, Lønborg & Søndergaard 2009).

Export to adjacent areas presents another important loss term for DOM in coastal areas. The percentage of degradable DOM that is consumed within a system is dependent on the water residence time of the system, affecting the oxygen consumption and nutrient loads of the respective and adjacent areas (cf. Søndergaard et al. 2004). Faster regeneration of N and P compared with C in DOM may lead to export of C-enriched DOM (Hopkinson et al. 2002, Lønborg et al. 2009). Recent investigations have demonstrated export of labile DOM (LDOM) out of coastal areas, implying a contribution to heterotrophic growth in adjacent areas (Lønborg et al. 2009, Lønborg & Søndergaard 2009).

1.2. Accumulation of DOM in marine surface waters and factors controlling bacterial consumption of DOM

DOM accumulates in various marine surface waters during stratified periods (e.g. Copin-Montégut & Avril 1993, Carlson et al. 1994, 2000, Williams 1995, Lønborg et al. 2009). The accumulating material is susceptible to export out of the surface layer to deep water via vertical diffusion and water-mixing events. Globally, this export of DOC from surface water and out of contact with the atmosphere is potentially a marked C sink that equals or even exceeds that of POM in many marine areas (e.g. Carlson et al. 1994, Emerson et al. 1997, Tian et al. 2004), but remains lower in others, such as the North Atlantic (9–20 % of the total C export; Carlson et al. 2010). One important feature of vertical DOM export is that it may occur with clearly higher C:N and C:P ratios than POM export, enabling more efficient recycling of nutrients in the surface water and making the C export more efficient per given amount of nutrients (Hopkinson & Vallino 2005).

Accumulation of autochthonous DOM in the surface waters occurs both during actively growing and decaying phytoplankton blooms as a result of decoupling of DOM release

and loss processes (e.g. Norrman et al. 1995, Søndergaard et al. 2000). Accumulation of DOC may stem either from low degradability of the accumulating material for the bacterial assemblage (e.g. Thingstad & Lignell 1997, Søndergaard et al. 2000, Carlson et al. 2002) or from incapability of the bacteria of consuming all degradable DOC, due to food web processes that control the biomass and growth of bacteria, i.e. a "malfunctioning microbial loop" (Thingstad et al. 1997). The form of bacterial growth limitation (C or nutrients) is closely linked with control of the net DOC pools (Thingstad & Lignell 1997, Carlson et al. 2002, Pinhassi et al. 2006, Thingstad et al. 2007). C limitation of the bacterial community prevents accumulation of LDOC, thus leading to lower accumulation and export of DOC than in situations where nutrients limit bacterial growth and allow accumulation of easily degradable DOM compounds (e.g. Thingstad et al. 1997, Thingstad & Lignell 1997, Carlson et al. 2002).

Whether bacterial growth is nutrient- or C-limited is dependent on the relative availability of inorganic nutrients vs. LDOC, competition for nutrients between bacteria and algae, and the nutrient requirements and adaptation to utilize specific DOM components of the bacterial assemblage (Thingstad & Lignell 1997, Cottrell & Kirchman 2000, Pinhassi et al. 2006). Both availability of LDOC (Kirchman & Rich 1997, Rivkin & Anderson 1997, Carlson et al. 2002) and inorganic nutrients (Rivkin & Anderson 1997, Sala et al. 2002, Pinhassi et al. 2006) limit bacterial growth in marine surface waters. Temperature may also function as an important regulator of bacterial growth and DOM consumption (e.g. Autio 1998, Zweifel 1999). It may affect the growth of the heterotrophic compartments of plankton systems more than algal growth, contributing thus potentially to the competition for nutrients between bacteria and algae (Pomeroy & Deibel 1986, Rose & Caron 2007, Thingstad et al. 2008). Several factors may limit bacterial growth simultaneously, e.g. LDOC availability and temperature (Kirchman et al. 2005) or LDOC and nutrients (Kuparinen & Heinänen 1993, Pinhassi et al. 2006). Grazing by HNF commonly controls bacterial biomass in marine surface waters (e.g. del Giorgio et al. 1996), and both experimental data and model simulations suggest that accumulation of LDOC may stem from combinations of nutrient- or temperature-limited bacterial growth with control of bacterial biomass by grazing (Thingstad & Lignell 1997, Zweifel 1999). The type and severity of bacterial growth limitation may show notable seasonal variation (Pinhassi et al. 2006).

1.3. Effects of photochemical transformation of DOM to bacterial growth

In addition to the biological processes, sunlight-induced photochemical transformations can markedly contribute to the turnover of the DOM pools in fresh and marine surface waters (Moran & Zepp 1997, Vähätalo 2009). The effects of solar radiation in an aquatic ecosystem are dependent on the amount and energy of the photons that reach the water surface, being thus affected by the latitude, season and time of day, as well as cloudiness, aerosols and atmospheric ozone content. When solar radiation enters the water surface, part of the radiation is reflected and the angular distribution of the photon flux changes. The optical properties of the medium modulate the attenuation of radiation in the water column.

Absorption of photons in the ultraviolet (UV) and short-wavelength visible light

regions of the solar spectrum has enough energy to initiate photochemical reactions. The energy of the photons decreases with increasing wavelength and thus reactions in the UV-B region (280–315 nm) of the solar spectrum are most effective in bringing about photochemical transformations. The amount of photons, however, increases with increasing wavelength and the short wavelengths attenuate more rapidly with depth, and photons of the UV-A (315–400 nm) and visible light (400–800 nm) regions of the spectrum thus are more of an influence at greater depths.

Coloured, chromophoric DOM (CDOM) dominates the absorption of UV radiation in many surface waters, contributing 90 % of the absorption of solar radiation at the UV-A range of the spectrum in the Baltic Sea (Babin et al. 2003). Thus, the concentration of CDOM largely determines the attenuation of UV radiation in the water column. These primary absorbers of solar radiation may act as sensitizers for further photochemical reactions that lead to transformation of molecules, such as most algal-derived DOM, which does not directly absorb radiation. Absorption of solar radiation may lead to direct photomineralization of DOM molecules, e.g. into CO_2 or CO, thus removing organic C from the surface system (e.g. Miller & Moran 1997, Moran & Zepp 1997, Vähätalo & Zepp 2005). Photochemical reactions contribute even more to removal of terrigenous and lake-water DOM than bacterial degradation (Obernosterer & Benner 2004). Algal-derived DOM is, in turn, less susceptible to photochemical degradation and is mainly degraded by bacteria (Thomas & Lara 1995, Obernosterer & Benner 2004).

Photochemical reactions also cleave DOM molecules into smaller organic compounds, such as fatty acids and keto acids, increasing the biological availability of initially refractory DOM (e.g. Miller & Moran 1997, Moran & Zepp 1997, Benner & Biddanda 1998). In addition to photochemical release of labile C substrates, photochemical transformation of humic DOM releases biologically available N as NH_4^+ (e.g. Bushaw et al. 1996, Vähätalo & Zepp 2005) and N-rich organic compounds such as amino acids (Jørgensen et al. 1998, Bushaw-Newton & Moran 1999). Photochemical transformation of DOM thus provides a source of new N in estuarine and coastal surface waters, the input of N by photoammonification being comparable to the atmospheric N load in the Baltic Sea in summer (Vähätalo & Zepp 2005). Labile photoproducts stimulate bacterial activity in coastal surface waters, leading to more complete decomposition of the DOM pool (Miller & Moran 1997, Moran & Zepp 1997, Bushaw-Newton & Moran 1999). Under N-limited conditions, photochemical release of labile N can also stimulate autotrophic production and biomass (Vähätalo & Järvinen 2007, Vähätalo et al. 2011). Photochemical production of biologically available DOM in coastal waters is potentially a notable sink of terrestrial DOM that could even equal riverine inputs of DOM (Miller et al. 2002).

Ambient labile bacterial substrates, in turn, are susceptible to photochemical transformation into more refractory compounds, which potentially decreases bacterial activity (Benner & Biddanda 1998, Tranvik & Kokalj 1998, Obernosterer et al. 1999). It has been suggested that photochemical transformation of DOM could contribute to the production of biologically refractory DOM that persists in the deep ocean for decades or more (Benner & Biddanda 1998). The effect of photochemical reactions on bioavailability of the DOM pool appears to be inversely related to its lability

for bacterial utilization before exposure (Obernosterer et al. 2001). In aquatic systems with large inputs of terrigenous DOM and in deep oceanic waters, the net effect of photochemical transformation of DOM on bacterial growth tends to be positive, whereas solar exposure of DOM from open-sea surface waters and of algal origin tends to decrease bacterial growth (Moran & Zepp 1997, Benner & Biddanda 1998, Tranvik & Kokalj 1998).

In addition to the effects of photochemical transformation of DOM on bacterial activity, UV radiation may affect bacterial growth directly. Interactions of photons with chemical bonds of living cells may modify the structure of their molecules, causing damage to deoxyribonucleic acid (DNA) and other molecules and inducing cell death and negative effects on growth. Radiation in the UV and visible light regions of the spectrum causes notable inhibition of bacterial production of both freshwater and marine bacteria (Sommaruga et al. 1997, Arrieta et al. 2000, Fernández Zenoff et al. 2006). Bacteria are more susceptible to UV damage than other microorganisms (Jeffrey et al. 1996).

1.4. Interactions of DOM pools and bacterial community composition

Modern genetic and molecular methods suggest that biologically available marine DOM is consumed by diverse bacterial communities (e.g. Pommier et al. 2007, Mou et al. 2008). Composition of the bacterial plankton assemblage may show relatively small variation over large spatial scales (Acinas et al. 1997), but differences in community composition occur with depth and even over small horizontal distances in areas where distinct water masses confront (Suzuki et al. 2001, Pinhassi et al. 2003, Herlemann et al. 2011). Seasonal variation in bacterial assemblages may be notable (Burkert et al. 2003, Schauer et al. 2003, Andersson et al. 2010), and the variability in activity of different groups of pelagic bacteria is even more dynamic than their relative contribution to the bacterial biomass (Alonso-Sáez & Gasol 2007).

The environmental factors and biogeochemical properties of oceanic water masses appear to control the global distribution of the major components of the marine bacterioplankton (Selje et al. 2004). Salinity may set limits on the growth of bacterial groups, and the availability of nutrients and LDOC can affect distinctly different bacterial groups within a community (Suzuki et al. 2001, Pinhassi & Berman 2003, Pinhassi et al. 2003, Andersson et al. 2010). The varying responses of bacterial subpopulations to nutrient amendments suggest that the limiting nutrient may not be the same for all bacterial groups within a community (Flaten et al. 2003). Grazing pressure and viral lysis represent other important selective forces that shape the composition of bacterial assemblages (Castberg et al. 2001, Gasol et al. 2002, Øvreås et al. 2003). Host-specific viral lysis may control bacterial diversity by selectively infecting the superior competitors, i.e. "killing the winner" (Thingstad and Lignell 1997, Thingstad 2000, Suttle 2007).

Growth responses to specific DOM compounds differ among bacterial groups, implying that the quality of the DOM pool markedly contributes to bacterial community composition (e.g. Cottrell & Kirchman 2000, Elifantz et al. 2007, Mou et al. 2007). The functional groups of bacteria that are responsible for utilization of the various components of the marine DOM pool are still largely unknown (e.g. Gasol et al. 2008,

Mou et al. 2008). Marine studies conducted below the whole-community level indicate some trends in utilization of different DOM components by broad phylogenetic bacterial groups. For example, α-Proteobacteria are, in various marine environments, proportionally more active in utilization of labile low-molecular-weight compounds, such as amino acids, than other major bacterial groups, whereas utilization of polymeric substances, such as proteins, chitin and extracellular polymeric substances may be dominated by Bacteroidetes bacteria (Cottrell & Kirchman 2000, Elifantz et al. 2005, Alonso-Sáez & Gasol 2007). γ-Proteobacteria may again respond quickly to increases in LDOM, such as glucose (Pinhassi & Berman 2003, Alonso-Sáez et al. 2009, Teira et al. 2010), and β-Proteobacteria dominate the degradation of humic substances in freshwater (Burkert et al. 2003).

Sunlight-induced photochemical transformation of the DOM pool may markedly affect bacterial community composition in freshwater and coastal areas (Judd et al. 2007, Perez & Sommaruga 2007, Abboudi et al. 2008, Piccini et al. 2009). Both positive and negative overall effects of photochemical transformation of DOM on bacterial growth have been accompanied by clear shifts in relative abundances of the major bacterial groups (Perez & Sommaruga 2007, Piccini et al. 2009).

Specific groups, genera and species within major bacterial groups may substantially differ in their responses to specific DOM compounds (Mou et al. 2007, Teira et al. 2009). In coastal environments with heterogenous DOM supplies, the consumption of ubiquitous DOM compounds appears to be dominated by large numbers of generalists across several major bacterial groups (Mou et al. 2008). Since no single group of bacteria dominates consumption of all DOM compounds, it appears that the contribution of a diverse bacterial assemblage is necessary for the degradation of complex DOM pools in marine environments (Cottrell & Kirchman 2000).

Community composition appears to affect functioning of the bacterial community (Kirchman et al. 2004, Teira et al. 2010). Distinct bacterial communities may respond to changes in DOM supply differently, with variation in their ectoenzyme production and possibly DOM mineralization capacities (Kirchman et al. 2004), which could explain the better availability of riverine DOM to estuarine than to limnic bacterial assemblages (Stepanauskas et al. 1999a, b, Wikner et al. 1999). However, various bacterial communities may also show similar functions, suggesting that this coupling between functioning and composition of bacterial communities is not always tight (Langenheder et al. 2005). The complexity of the DOM pools and wide taxonomic diversity of bacterial communities impede the understanding of interactions between bacterial groups and community functioning, and thus further exploration of these linkages is needed for predictive modelling of C cycling in a changing ocean (e.g. Gasol et al. 2008, Mou et al. 2008, Teira et al. 2010)

2. AIMS AND INVESTIGATIONS OF THE STUDY

DOM pools overwhelmingly dominate aquatic C and nutrient stocks, and this thesis was conducted to improve the insight into the role of these pools in the dynamics of the plankton system and cycling of C and nutrients in the northern Baltic Sea. Special emphasis was given to the interaction between heterotrophic bacteria and the DOM pool.

The balance between supply and loss processes determines the ambient net pools of DOM. In this thesis, the importance of DOM pools to the planktonic ecosystem and nutrient cycling in the Gulf of Finland (GoF) were examined. One aim was to follow the seasonal dynamics of the net DOM pools and their stoichiometry (C:N:P ratios; I, III). The biological availability of DOC and DON were investigated to determine the role of the ambient DOM pools in nutrition of the planktonic assemblage (I, III). Intensive phytoplankton blooms occur in the eutrophicated Baltic Sea, and the question was addressed as to how they affect the prevailing DOM pools with large background percentages of humic substances of terrestrial origin (III, this thesis). The composition of the DOM pool, which potentially may affect bacterial functions and community composition and remains mostly unknown in the Baltic Sea, was beyond the scope of this thesis.

Another aim of the thesis was to improve the view of several aspects on key loss processes of net DOM pools, including biological and photochemical degradation and physical transport. Thus, factors that limit bacterial growth and degradation of the DOM pools in both surface and deep water were investigated during different stages of the productive season (I, II). Moreover, the effects of sunlight-induced photochemical transformation of DOM on bacterial growth and thus degradation of DOM were estimated (I, II, IV). Since photochemical reactions may alter both the quantity and quality of the DOM pool, the effects of photoproduced LDOM on the composition of the natural bacterial assemblage were also addressed (IV). The results of these investigations form a coherent view of the ambient net DOM pools as storage areas of phytoplankton-derived DOM and as a component of C flow and nutrient cycling in the GoF.

3. STUDY AREA

These studies were conducted in the coastal and open-sea areas of the GoF (Fig. 1), which is situated in the NE part of the Baltic Sea. The Baltic Sea is one of the world's largest brackish water basins, with a surface area of 377 000 km^2 and average depth of 55 m. The GoF is directly connected to the Baltic

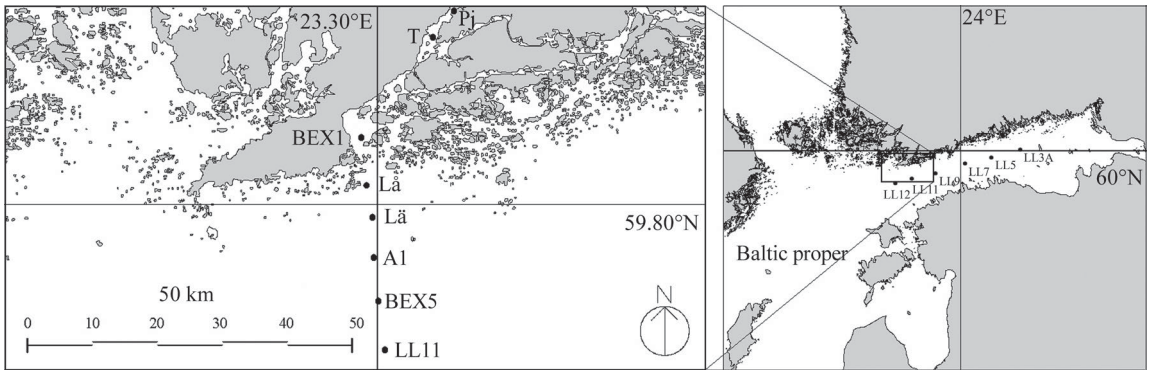


Fig. 1. Sampling sites. Most of the studies were conducted with sample water from the outer archipelago and open-sea areas in the W GoF (Lå–LL11). A1 = Ajax1, Lå = Långskär, Lä = Längden, Pj = Pojo and T = Tammi.

Proper with no separating shallows. It is influenced by deep water of the Baltic Sea and freshwater inflow, largely from the eastern part of the GoF. The freshwater balance of the GoF is positive, and the basic surface circulation off the coast of Finland flows west towards the mouth of the GoF, due to the Coriolis force. The renewal time of the water mass in the gulf is about five years (Andrejev et al. 2004). The Neva, the largest river in the Baltic Sea catchment area, flows to the eastern end of the GoF and strongly influences its hydrography, creating a horizontal salinity gradient. The salinity in the open-sea surface water of the study area ranges from 4 at the easternmost study site to 6 in the W GoF. The salinity in the deep water is approx. 7. The concentrations of total N and P, particulate organic N (PON) and P (POP) and dissolved inorganic N (DIN) and P (DIP) all decreased from the eastern to the western parts of the GoF (Pitkänen et al. 1993, Perttilä et al. 1995, Kuuppo et al. 2006).

The main study area in the W GoF was chosen because it is mainly influenced by the surface water flow of the GoF. The surface outflow from Pojo Bay does not reach our open-sea GoF stations outside the Långskär site (Längden-LL11; Fig. 1) (Niemi 1975). The water residence time in this area is about one year (Andrejev et al. 2004). For examination of the DOM dynamics on a salinity gradient, samples were also collected from the freshwater end and middle of fjord like Pojo Bay and from the archipelago (BEX1; Fig. 1). BEX1 shows notable fluctuations in salinity and temperature, due to mixing of the water masses originating from the seaward flow of oligohaline water from Pojo Bay, the open-sea surface water and intrusions of deep water extending from Ajax1 to BEX1 along the deep furrow in the bottom topography (Niemi 1975).

In the GoF, temperature stratification develops in May and the water column remains stratified, with a thermocline at depths of 10–15 m for the entire summer, until cooling temperatures and strong winds induce mixing of the water column during the autumn overturn. The spring bloom emerges in April–May, with dominance of diatoms and dinoflagellates (e.g. III, Niemi 1975). The spring bloom exhausts the DIN from the surface layer (III, Niemi 1975, Lignell et al. 1992, 2003). After decay of the spring bloom, the summer minimum period begins (approx. June– mid-July) with low phytoplankton biomass, which is dominated by pico- and nanophytoplankton. Since the 1990s, excess PO_4^{3-} has remained in the surface layer after decay of the spring bloom, and the low inorganic N:P ratios during the summer minimum period have suggested N-limitation of the phytoplankton community (III, Lignell et al. 2003). In mid-July– August, a bloom of diazotrophic cyanobacteria emerges, leading to depletion of the ambient PO_4^{3-} pool and the plankton system turns towards combined N and P (NP) limitation (III, Lignell et al. 2003). Below the thermocline, cold temperatures and higher inorganic nutrient concentrations (PO_4^{3-} and NO_3^-) prevail (Niemi 1975, Laanemets et al. 2004). During the stratified period, frequent upwellings during moderate SW–NW winds introduce new nutrients in to the surface layer in the main study area of the W GoF.

In the GoF, annual primary production ranges from 6 to 9 mol C m^{-2} and the annual net bacterial production is estimated to range from 10 % to 15 % of the primary production (Lignell 1990 and references therein). The ratio of bacterial C demand (BCD) to primary production is 0.5–0.6, suggesting that the BCD could be supported by autochthonous production (Hagström et al. 2001).

4. MATERIALS AND METHODS

4.1. Monitoring seasonal dynamics of DOM (III)

To follow the dynamics of the various constituents of the DOM pool, seven sites on a transect from a river mouth to the open sea (Pojo-LL11; Långskär not included) in the W GoF were sampled biweekly from early April to mid-September 2002 (Fig. 1). At the Pojo Bay and Tammisaari stations, surface water from a depth of 2 m was collected, whereas in the archipelago (BEX1) and open-sea (Längden-LL11) areas the surface layer was sampled for DOC and DON analysis every 2.5 m down to a depth of 15 m while below that the deep layer was sampled every 10 m as described in III. DOP and LDOM (4.2.3) were determined from the pooled surface (0–10 m) and deep-water (> 20 m) samples, as described in III. To determine the factors controlling the net pools of the various DOM constituents, key physical (temperature, salinity, fluorescence, secchi depth), chemical (chlorophyll-*a* (chl-*a*), inorganic nutrients, CDOM; representing humic substances) and biological (bacterial biomass and phytoplankton diversity and biomass) background factors were measured, as described in III. The five archipelago and open-sea sites on the shore-to-open-sea transect (BEX1, Längden, Ajax1, BEX5, LL11) were additionally sampled for DOC and DON analysis on 14–16 January 2002, and the horizontal coverage of the DOC and DON data was supplemented with sampling of six sites on an E–W transect on 30 July–1 August 2001 and 14–16 January 2002, as described in III (Fig. 1).

4.2. Experimental studies (I–IV)

The experimental studies presented in this thesis were mainly carried out in the W GoF (at the Tvärminne Zoological Station, University of Helsinki) in 2001–2005 (Fig. 1). In summer 2001 (I) and January 2002 (III) some of the experiments were conducted during a cruise on an E-W transect of the GoF. Pooled surface (0–10 m) and deep-water (> 20 m) samples were collected at the outer archipelago and open-sea sites and surface water from 2-m depths in Pojo Bay, as described in I–IV.

4.2.1. Accumulation of DON during a late summer cyanobacterial bloom (this thesis)
The time courses of DON were followed in a mesocosm experiment conducted in the inner archipelago (site Storfjärden) off the W GoF from 1 to 22 July in 2003. The experimental setup is described in Kangro et al. (2007). Briefly, a natural water column was enclosed in nine bags with volume of 51 m^3. Of the four mesocosms discussed in this thesis, three received additions of N (1 μmol NH_4^+-N l^{-1} d^{-1}) and P (1/16 μmol PO_4^{3-}-P l^{-1} d^{-1}) for a 5-day boosting period, after which one mesocosm (P) continued receiving the same P addition, and the other two boosted mesocosms (5P and 5PG) received five-fold the P addition. The mesocosm 5PG also received glucose (13.3 μmol C l^{-1} d^{-1}) after the boosting period. The fourth mesocosm (control) received no nutrient additions. Samples for DOC and DON analysis were taken every other day.

4.2.2. Effects of inorganic nutrients and glucose-C on bacterial growth and exploitation of DOC and DON (I)

To examine the effects of inorganic nutrients (N, P) and glucose-C on bacterial growth and exploitation of DOC and DON, three bacterial incubation experiments were conducted during the summer minimum period (14 June and 4 July 2001) and late summer cyanobacterial bloom (17 July 2002) with open-sea water from the W GoF. The experiments were designed to create extreme C-limited (NP treatment) and N-limited (P treatment) conditions to maximise bacterial degradation of the LDOC and LDON pools (Table 1). Samples containing natural bacterial assemblages were prepared, treated with different nutrient additions (Table 1) and incubated for 2–3 weeks, as described in I.

4.2.3. Spatial and seasonal variation in LDOC and LDON pools (I, III, this thesis)

For experiments on seasonal variation in LDOC and LDON pools, pooled surface and deep-water samples were collected from the outermost open-sea site (LL11) in the W GoF and surface samples from the river mouth of Pojo Bay biweekly from early April to mid-September 2002 (III; Fig. 1). Sample water was exceptionally collected closer to the shore on 3 July (Långskär) and 23 September (Längden), due to strong SW and W–NW winds, respectively. The spatial coverage of the experiments was supplemented with samples from three to five sites along an E-W transect on 30 July–1 August 2001 and 14–16 January 2002 (I, this thesis). The samples for the LDOM experiments were collected simultaneously with the collection of DOC and DON samples for the field study.

Table 1. Experimental design of factors controlling bacterial growth and degradation of dissolved organic matter (DOM) in the surface and deep water of the W GoF in 2001 and 2002. Natural bacterial samples (< GF/F filtrate) were treated with C, nutrients and heterotrophic nanoflagellates (HNF): N = 7.1 μmol NH_4^+- N l^{-1}; P = 1.4 μmol PO_4^{3+}- P l^{-1}; C = 83 μmol glucose-C l^{-1}; Control = no nutrient or C additions; Flag = <5-μm inoculum including HNF (10% vol/ vol); +/- = treatment carried or not (five replicate 1-l bottles per treatment). In 2002, the experiments were conducted biweekly.

Treatment	14 Jun 2001 Surface water	4 Jul 2001 Surface water	9 Apr- 25 Sep 2002 Surface water	9 Apr- 25- Sep 2002 Deep water
Control	-	-	+	+
N	-	-	+	-
P	-	+	+	-
C	-	-	+	-
NP	+[1)]	+	+	+
PC	-	+	+	-
NP+C	+	+	-	-
Flag	-	-	+	-
NP+Flag	-	+	-	-

[1)] <0.2-μm filtered sample with <0.8-μm inoculum

Five replicate bacterial samples (< 0.7-μm filtering; GF/F glass fibre filters) were prepared as described in I and III and treated with different nutrient combinations, according to Table 1. For the samples from Pojo Bay and the E-W transect only NP treatment was conducted. The samples were incubated in the dark at an *in situ* temperature for 2 weeks and the DOC and DON concentrations were measured at the start (day 0), on day 2–3 (only NP-treated samples of 2002) and at the end of the incubations. In January, the incubation time was 3 weeks and some of the surface samples were incubated at an *in situ* temperature (3 °C) and some at an elevated temperature (12 °C).

4.2.4. Changes in carbon and nutrient availability and temperature as factors controlling bacterial growth (II)

The effects of inorganic nutrients (N and P) and glucose-C treatments on bacterial growth were followed for 3 days in natural surface (0–10 m) and deep-water (20–40 m) bacterial samples in the W GoF. The samples were taken on 12 May, 9 June, 1 July and 11 August 2003. The samples were generally collected from an open-sea site (Ajax 1), but in July pooled surface (0–7 m) samples from the inner archipelago (Storfjärden) were used. The samples were prepared and treated with nutrients (glucose-C, NH_4^+-N, PO_4^{3-}-P), following a complete 2^3 factorial design with all eight combinations of duplicated treatments, as described in II. For deep-water samples, correspondingly a 2^2 factorial design was used with glucose-C and the NP treatments. Surface samples were incubated in the dark for 3 days, as described in II. In May and June, the incubation temperatures were elevated from the low *in situ* temperatures of 3–6 °C and 6–9 °C to 10 °C and 16 °C, respectively. In July and August, an *in situ* temperature of 18 °C was used. Deep-water samples were incubated at the same temperature with corresponding surface samples in May and June, but in August at 13 °C (II). To examine the effect of temperature on growth of deep-water bacteria, control and NPC-treated deep-water samples were also incubated at an *in situ* temperature of 3 °C.

4.2.5. Effects of photochemical transformation of DOM on bacterial growth (I, II)

The importance of photochemical transformation of DOM on bacterial growth was examined with a series of 1-day sunlight pretreatment experiments with subsequent incubations with natural bacterial assemblages in 2001 and 2003. In 2001, surface water samples for the experiments were collected from Längden (W GoF) on 4 July and from three sites along an E-W transect on the GoF (LL7, LL3A, XV1) on 30 July–1 August. In 2003, the horizontal and seasonal variations in the effects of DOM photoproducts on bacterial growth were assessed with a four-experiment series that was conducted with surface samples from the river mouth (Pojo), archipelago (about 2 km south from Långskär) and open-sea (Ajax1) sites in the W GoF. The samples were collected on 12 May, 9 June, 7 July and 11 August. They were prepared, exposed over 1 day to natural sunlight in quartz bottles (with aluminium foil-wrapped dark samples as controls) at depths of 0.2 m (I) or 0.1, 0.3, 0.7 and 2 m (II) and subsequently treated with either N and P or no nutrients and incubated for 5–8 days with 10 % (vol/vol) natural inocula containing bacteria or bacteria and HNF (Table 2) as described in I and II.

Table 2. Experimental design of the effects of photochemical transformation of DOM on bacterial growth in the surface water of the W GoF in 2001 and 2003. Particle-free samples (<0.2 µm) were exposed to natural sunlight for 1 day (dark controls wrapped in aluminium foil) and treated with nutrients and heterotrophic nanoflagellates: N = 7.1 µmol NH_4^+- N l^{-1}; P = 1.4 µmol PO_4^{3+}- P l^{-1}; C = 83 µmol glucose-C l^{-1}; None = no nutrient or C additions; Flag = <5-µm inoculum including HNF (10% vol/ vol); +/- = treatment carried or not (three or five replicate 250 ml bottles per treatment).

Treatment/ exposure depth	4 Jul 2001 0.2 m	30 Jul–1 Aug 2001 0.2 m	May–Aug 2003 0.1 m	0.3 m, 0.7 m, 2 m
None	+	-	+	-
NP	+	+	+	+
Flag	+	-	-	-

4.2.6. Effects of photochemical transformation of humic refractory DOM on microbial growth and community composition (IV)

For the study on the effects of photochemical transformation of refractory DOM on growth and composition of the microbial community, including bacteria, algae and small protists (< 10 μm), surface water (0–5 m) from Långskär was collected on 15 July 2005. Firstly, an indigenous plankton inoculum (< 10 μm) was treated with PO_4^{3-} and incubated for 6 days, as described in IV, to remove biologically labile C and N (pretreatment). The pretreated sample water was then filtered through 0.2-μm filter and exposed in quartz bottles to ambient solar radiation (with aluminium foil wrapped dark samples as controls) for 14 days at an *in situ* temperature in a matte black outdoor pool flushed with tap water, as described in IV. For the bioassay, the sunlight-exposed and the dark control waters were inoculated with a natural plankton inoculum (< 10 μm, 10 % vol/vol), in which the number of filamentous cyanobacteria had been reduced, and incubated at an *in situ* temperature under photosynthetically active radiation (PAR) for 10 days, as described in IV.

4.3. Contamination precautions

To avoid contamination, the procedures outlined by Sharp et al. (1993) were followed with slight modifications. Briefly, all quartz- and glassware, glass fibre filters and polycarbonate bottles were placed for at least 2 hours in 15 % HCl and subsequently rinsed carefully with tap water, Milli-rho and Milli-Q water (EMD Millipore Corp., Billerica, MA, USA). In addition, quartz glass bottles, glassware and glass-fibre filters were heated to 400 °C for at least 4 hours. All containers, filters and sample bottles were thoroughly rinsed with sample water before use.

4.4. Measurements

The parameters followed in this thesis were measured with previously published methods, summarized in Table 3, as described in I–IV.

Table 3. Summary of the methods used in analyzing the samples in I-IV. Analyses were conducted by: 1 = authors of I–IV with notable contribution by the author of the thesis, 2 = other authors of I–IV, 3-5 = Laboratories of 3) the Tvärminne Zoological Station, 4) the Finnish Institute of Marine Research and 5) the Lammi Biological Station, 6) K. Kivi or H. Kuosa, - = analysis not conducted.

	Parameter	Method	References	I	II	III	IV
Water chemistry	NH_4^+	Phenolhypochlorite method	Grasshoff et al. 1983	3	3	3,4	2
	1) NO_3^- and NO_2^-	Reduction of NO_3^- to NO_2^- and colorimetric determination of the NO_2^-	Grasshoff et al. 1983/ Lachat QuikChem method 31-107-04-1-A	3	3	3,4	2
	Soluble reactive P (SRP)	Colorimetric determination with molybdate method	Grasshoff et al. 1983/ Lachat QuikChem method 31-115-01-3-A	3	3	3,4	4
	Total dissolved P (TDP) and dissolved organic P (DOP)	Colorimetric determination after persulphate oxidation, DOP = TDP - SRP	Koroleff 1979	-	-	3,4	4
	Dissolved organic C, total dissolved N (TDN) and dissolved organic N (DON)	High temperature catalytic oxidation (Shimadzu TOC-V_{CPH}), DON = TDN – ($NO_{2/3}^-$ + NH_4^+)	Sharp et al. 1993	1	1,3	1,3	1,3
	Particulate organic C	Filtration on GF/F glass fibre filters and determination with mass spectrophotometer (Europa Scientific ANCA-MS 20-20)	Salonen 1979	-	-	-	5
	Particulate organic N and P	Filtration on GF/F glass fibre filters, measurement as $NO_{2/3}^-$ and SRP after alkaline persulphate oxidation	Lachat QuikChem methods 10-115-01-1-F and 10-107-04-1I	-	-	-	5
	Total N and P	Colorimetric determination after persulphate oxidation	Koroleff 1976	3	-	-	-
	Chromophoric DOM	Spectrophotometric detection (Shimadzu UV-1201 PC)	Bricaud et al. 1981	-	1	1	2
	Chlorophyll-a	Spectrofluorometric detection (Shimadzu RF-5000)	Jespersen and Cristoffersen 1987	-	2	2	2
Bacteria	Abundance	Counting with epifluorescence microscopy of acridine orange stained cells	Hobbie et al. 1977	1	1	1	2
	Cell volume	Image analysis	Massana et al. 1997	1	1	1	2
	Bacterial production	Thymidine/ leucine incorporation	Fuhrman and Azam 1980, 1982 Smith and Azam 1992	1/-	1/-	-/-	1/1
	Community composition	Filter PCR Denaturing gradient gel electrophoresis Cloning	Kirchman et al 2001 Muyzer et al. 1995, Schauer et al 2000, 2003 Proced. of TOPO TA cloning kit	-	-	-	1
Phytoplankton and protists	Biomass of heterotrophic nanoflagellates (HNF)/ small sized autotrophs	Counting with epifluorescence microscopy of proflavine stained cells, HNF vol. with New Porton grid, C conv. 0.22 pg C μm^{-3} Cell vol. and C contents for autotrophs from HELCOM	Haas 1982 Børsheim and Bratbak 1987 HELCOM PEG Biovolume reporting 2008	1/-	1/-	1/-	2/2
	Biomass of large sized (>2 µm) autotrophs/ Ciliates	Counting with phase contrast microscopy of Lugol's solution stained cells C conv. for ciliates 0.19 pg C μm^{-3} and for autotrophs 0.11 pg C μm^{-3} (I-III) or from HELCOM (IV)	Utermöhl 1958 Putt and Stoecker 1989 HELCOM PEG Biovolume reporting 2008	6/-	6/-	6/-	2/2
	Primary production	^{14}C method	Niemi et al. 1983	-	-	-	2

4.5. Statistical examinations

All experiments conducted in this thesis were performed quantitatively, using statistical tests described in I–IV to extract significant treatment responses. The labile percentages of ambient DOC and DON pools within each treatment set in the experiments conducted in 2001 were determined from linear regressions fitted to the initial about 1-week declining periods of DOC and DON (I; five replicates, daily measurements). The LDOC and LDON pools were then calculated, using the slope of the regression line. The stoichiometry of the changes in the DOM pools in 2002 was determined from the slopes of the linear regression lines in element-element (DOC versus DON, DOC versus DOP and DON versus DOP) plots (cf. Hopkinson & Vallino 2005), using all average surface and deep-water DOM values (January–September) across the shore-to-open-sea transect (BEX1-LL11; Fig. 1; III).

The relationships between the various constituents of DOM (DOC, DON, DOP, LDOC, LDON) and key physical (temperature, salinity, CDOM absorption), chemical (inorganic nutrients) and biological (biomass of bacteria and phytoplankton) parameters in open-sea surface and deep water in 2002 were assessed by redundancy analysis (RDA), which is a direct ordination method with a linear response model, as described in III. RDA enables simplification of the model by sorting out significant explanatory variables (separate and marginal significances of each variable were taken into account; analysis of variance). Site- and time-related changes in chemical (DOC, DON, inorganic nutrients) and physical (temperature, salinity, fluorescence) water properties on the shore-to-open-sea transect were followed in further detail for different stages of planktonic succession (spring bloom, summer minimum, cyanoacterial bloom and late summer–autumn) with principal component analysis (PCA), as described in III.

To define intersample distances in the bacterial communities during the bioassay in 2005 (IV), the presence or absence of the bands and their relative intensities in each lane of the denaturing gradient gel electrophoresis (DGGE) gel were used to build a population matrix. The population matrix was then examined with nonmetric multidimensional scaling (NMDS), as described in IV.

4.6. Apparent quantum yield for stimulated bacterial production and rate of bacterial production based on photoproduced LDOM (IV)

In the study on the effects of photochemical transformation of refractory DOM on the microbial community, conducted in late summer 2005, bacterial C production at the expense of photoproduced LDOM was related to the number of photons absorbed during exposure, i.e. the apparent quantum yield ($\varphi_{bp,\lambda}$), as described in Vähätalo et al. (2011). The rate of bacterial production based on photoproduced LDOM at a depth of 0 m was then calculated, using the product of average dose of daily summer solar radiation modelled in Kuivikko et al. (2007), the measured absorption by CDOM and the estimated $\varphi_{bp,\lambda}$.

5. RESULTS

5.1. Seasonal dynamics of DOM (III)

During the study period (2001–2005) DOC concentrations varied between 290 and 430 μmol C l^{-1} in the archipelago and open-sea water of the W GoF (Fig. 2, Table 4), and between 540 and 720 μmol C l^{-1} in Pojo Bay (data not shown). The corresponding values for DON were 8.6 and 22.8 μmol N l^{-1} (shore-to-open-sea; Fig. 2, Table 4) and 10.7–38.5 μmol N l^{-1} (Pojo Bay; data not shown). The average surface water DOC and DON concentrations in the W GoF showed low yearly variation (Fig. 2, Table 4).

After formation of the temperature stratification (thermocline at depths between 10 and15 m) by late April 2002 (Fig. 3), both the DOC and DON began to accumulate in the surface water across the shore-to-open-sea transect with notable concentration increases, coinciding with the phytoplankton blooms (Figs. 2, 4). In late April, during the spring bloom dominated by dinoflagellates and diatoms, the DOC and DON concentrations (mean ± SD) reached values of 26 ± 14 μmol C l^{-1} (7 %) and 4.0 ± 1.0 μmol N l^{-1} (27 %) above the winter level (364 ± 9 μmol C l^{-1} and 12.8 ± 0.5 μmol N l^{-1} in mid-January), respectively. In mid-July during the cyanobacterial bloom, consisting mostly of flagellates and filamentous cyanobacteria with *Aphanizomenon flos-aquae* and *Nodularia spumigena* as dominant species, the DOC and DON concentrations were in turn 37 ± 14 μmol C l^{-1} (10 %) and 5.7 ± 1.1 μmol N l^{-1} (38 %) above the winter level, respectively.

Some local short-term changes in surface DOM concentrations consisted, at least in part, of allochthonous DOM that was transported from the surrounding areas. In the surface water in the archipelago (BEX1), the DOC concentration increased from the winter level by 7 % by early April (Fig. 2A), coinciding with a strong outflow from Pojo Bay, as suggested by a salinity minimum (Fig. 3C) and a subsequent peak in *in vivo* fluorescence (Fig. 4A). On 17 June, the DOC and DON concentrations in open-sea surface water (site LL11) diverged in turn from those of previous samplings, coinciding with a temporary salinity minimum (Figs. 2, 3). These open-sea peak DOC values were probably induced by low-salinity water masses from Pojo Bay or even farther north on the SW coast line of Finland, introduced by a switch in the locally predominant S–SW winds to persistent (1 week) moderate – strong N–NW winds (T. Stipa, pers. comm.; III).

Variation in the DOC and DON concentrations along the E-W transect in the GoF was monitored in August 2001 and in January 2002. The surface water DOC and DON concentrations were higher during summer than during winter across the E-W transect, showing that the changes in the DOM pools were not site-specific, but a general phenomenon in the GoF (III). The concentration of DOC in the surface water decreased by 40–100 μmol C l^{-1} from the E to the W GoF both in August and in January (III). The DON concentration decreased by approx. 1 μmol C l^{-1} from the E to the W GoF in August, but maintained even levels across this transect in January.

The DOP concentration in open-sea surface water doubled from late April to autumn (Table 5). Concomitantly, the DOC:DON, DOC:DOP and DON:DOP ratios decreased from spring to autumn. The stoichiometry of the vertical, horizontal and temporal variations in the total DOM pools was determined from the slopes of the linear

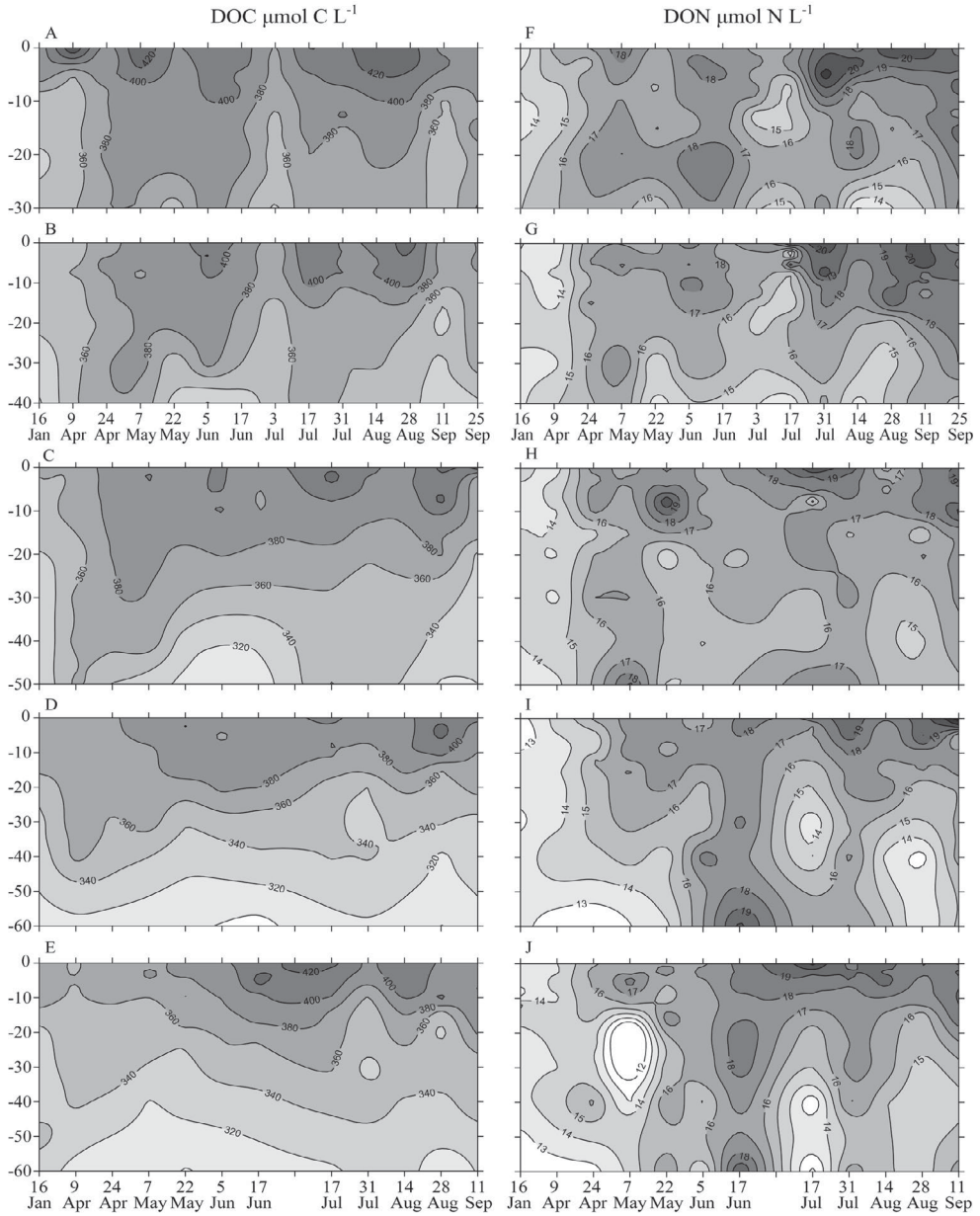


Fig. 2. Time courses for DOM concentrations at the five sampling sites on the shore-to-open-sea transect (BEX1, Längden, Ajax1, BEX5, LL11; Fig. 1) in the Gulf of Finland in January and during the productive season in 2002. Vertical profiles of DOC (A–E) and DON (F–J) from BEX1 (A, F) to LL11 (E, J). Note that the first sampling was conducted on 16 January and the second on 9 April, after which the sampling interval is approx. 2 weeks. Only the two sites closest to shore were sampled on 3 July and 25 September, due to a strong SW–W wind. However, during SW winds the sites Långskär and Längden present the same water mass, with hydrography similar to that of our open-sea sites. Figure redrawn from III.

Table 4. The ambient physical and chemical characteristics and the biomass of large sized (> 2 µm) phytoplankton with the share of cyanobacteria of the total phytoplankton biomass in the Gulf of Finland during the samplings for experimental studies. S= Surface (pooled sample 0-10 m, except for Pojo (Pj) 2 m), D= deep (pooled sample > 20 m). ND= not detected.

Period	Date	Site	Surface/ deep	Sal.	Temp. °C	DIN µmol N l^{-1}	SRP µmol P l^{-1}	DIN:SRP (mol mol^{-1})	DOC µmol C l^{-1}	DON µmol N l^{-1}	Phytopl. µmol C l^{-1} (cyanobacteria)	Secchi m
Summer minimum	14 Jun 2001	W archip. (Lå)	S	ND	10	0.40	0.24	1.7	383	19	9 (0 %)	8.0
	4 Jul 2001	W o-s (Lä)	S	ND	13	0.32	0.14	2.2	383	18	13 (14 %)	6.9
Cyanobacterial bloom	30 Jul 2001	E o-s (LL3A)	S	4.4	21	0.32	0.00	97	475	22	14 (85 %)	ND
	31 Jul 2001	E archip. (XV1)	S	4.1	19	1.14	0.01	88	483	22	4 (40 %)	ND
	1 Aug 2001	W o-s (LL7)	S	5.2	18	0.21	0.00	66	433	21	8 (72 %)	ND
Post springbloom	12 May 2003	W o-s (A1)	S	6.4	3	0.29	0.54	0.6	350	15	9 (6 %)	5.8
		W o-s (A1)	D	7.0	2	1.98	0.77	2.6	333	16	-	-
Summer minimum	9 Jun 2003	W bay (Pj)	S	ND	16	99.33	0.07	1338	633	36	ND	ND
		W archip. (Lå)	S	6.0	8	0.17	0.37	0.5	350	17	1 (3 %)	6.4
		W o-s (A1)	S	6.0	8	0.29	0.44	0.7	350	16	1 (4 %)	6.2
		W o-s (A1)	D	8.1	2	4.21	1.48	2.9	308	14	-	-
	1 Jul 2003	W archip.(BEX1)	S	5.8	15	0.15	0.22	0.7	383	18	3 (30 %)	ND
Cyanobacterial bloom	7 Jul 2003	W archip. (Lå)	S	5.8	16	0.95	0.22	4.4	408	19	6 (66 %)	3.5
		W o-s (A1)	S	6.0	15	1.29	0.26	4.9	375	17	5 (42 %)	6.5
	11 Aug 2003	W bay (Pj)	S	ND	20	9.70	0.27	35.3	716	33	ND	ND
		W archip. (Lå)	S	5.8	17	0.06	0.00	17.7	408	19	14 (29 %)	5.1
		W o-s (A1)	S	5.7	18	0.05	0.00	15.5	408	20	7 (33 %)	5.3
		W o-s (A1)	D	7.1	2	5.41	2.23	2.4	316	16	-	-
Cyanobacterial bloom	15 Jul 2005	W archip. (Lå)	S	5.3	18	0.28	0.10	2.8	418	17	18 (47 %)[1)]	ND

[1)] Small sized phytoplankton included

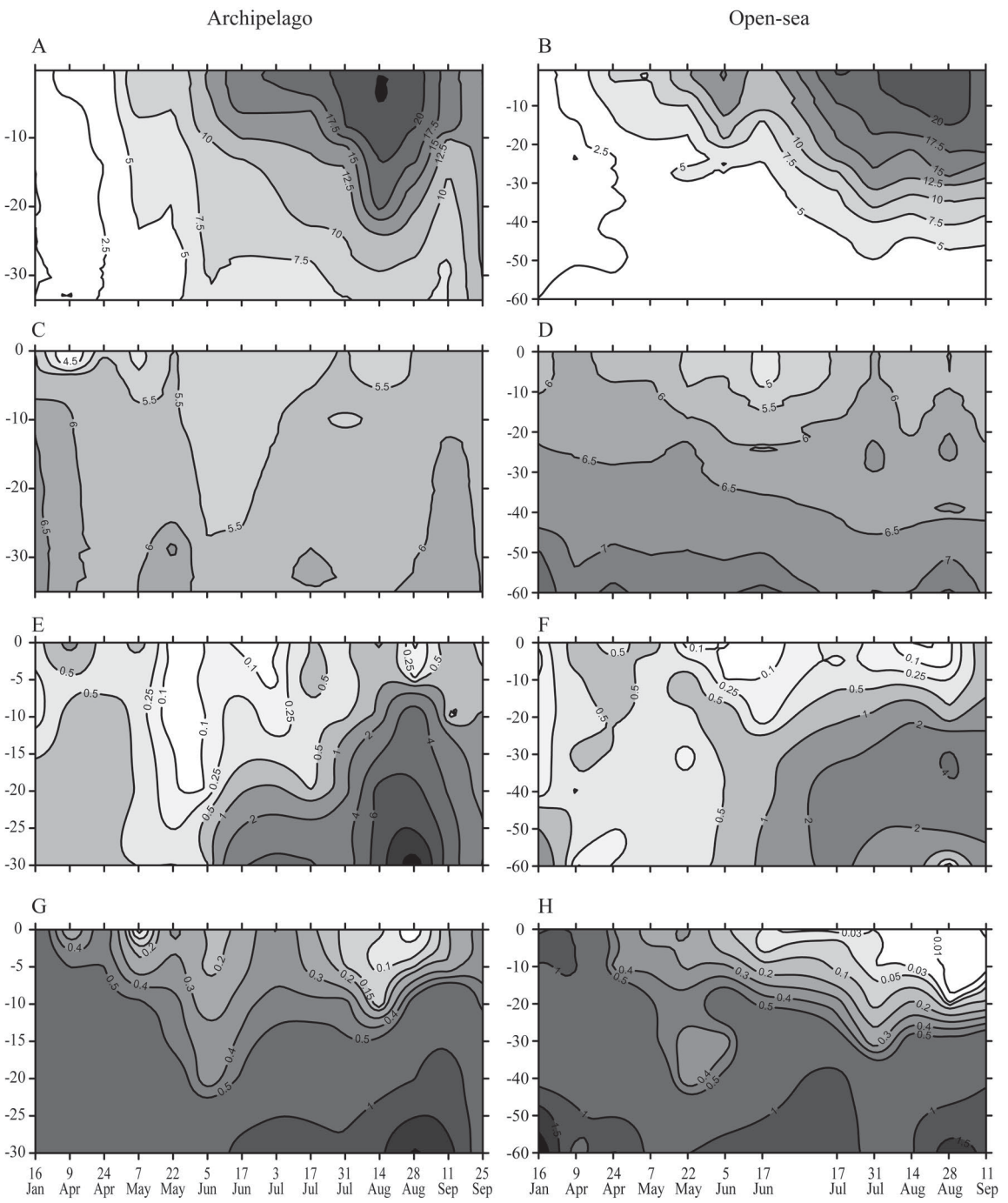


Fig. 3. Hydrography at archipelago (BEX1) and outermost open-sea (LL11) sites in the Gulf of Finland in January and during the productive season in 2002. Vertical profiles of temperature (A, B), salinity (C, D), and concentrations of NH_4^+ (E, F) and soluble reactive phosphorus (SRP; G, H) are shown. Note that the first sampling was conducted on 16 January and the second on 9 April, after which the sampling interval is approx. 2 weeks. Figure redrawn from III.

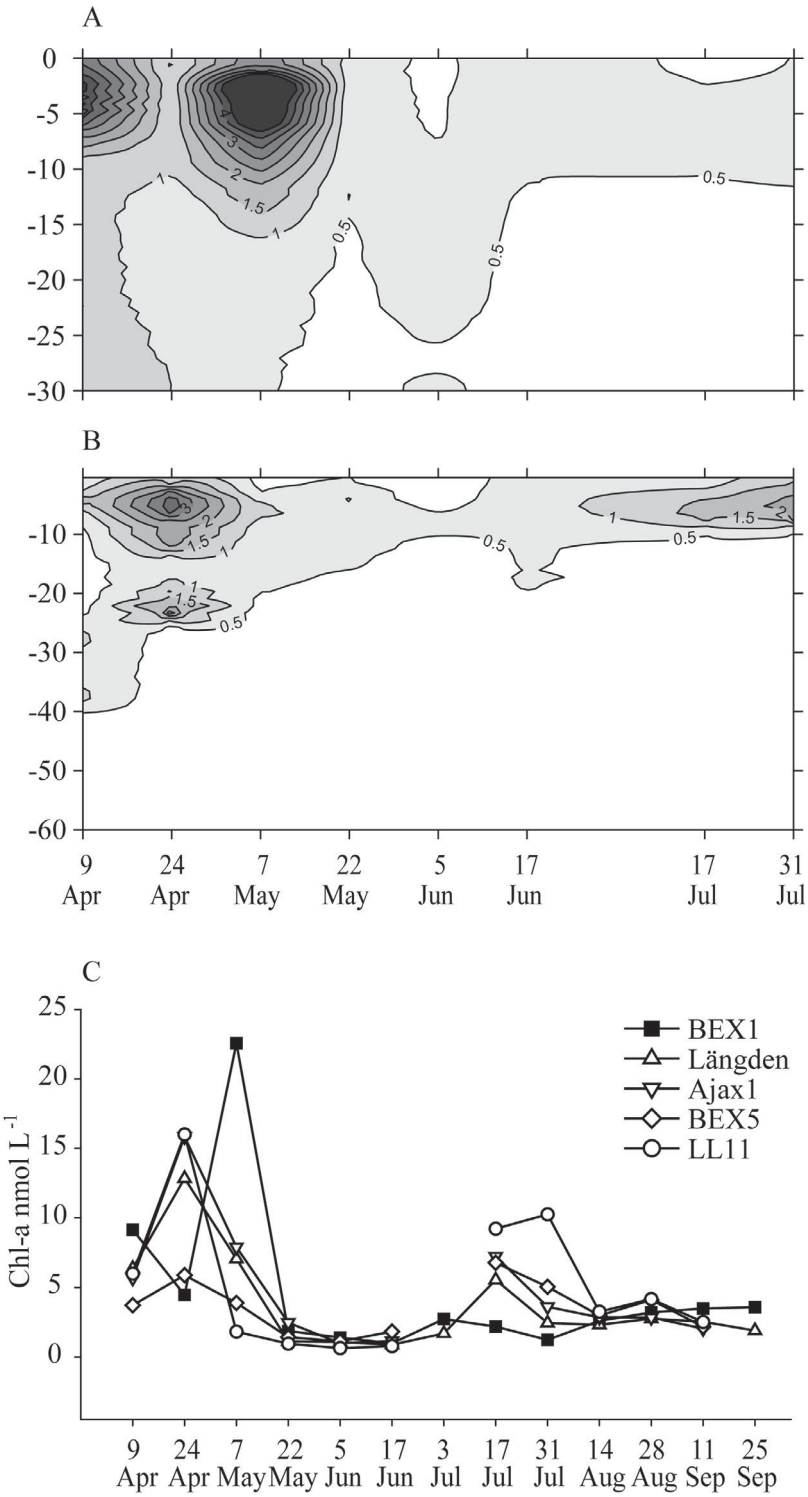
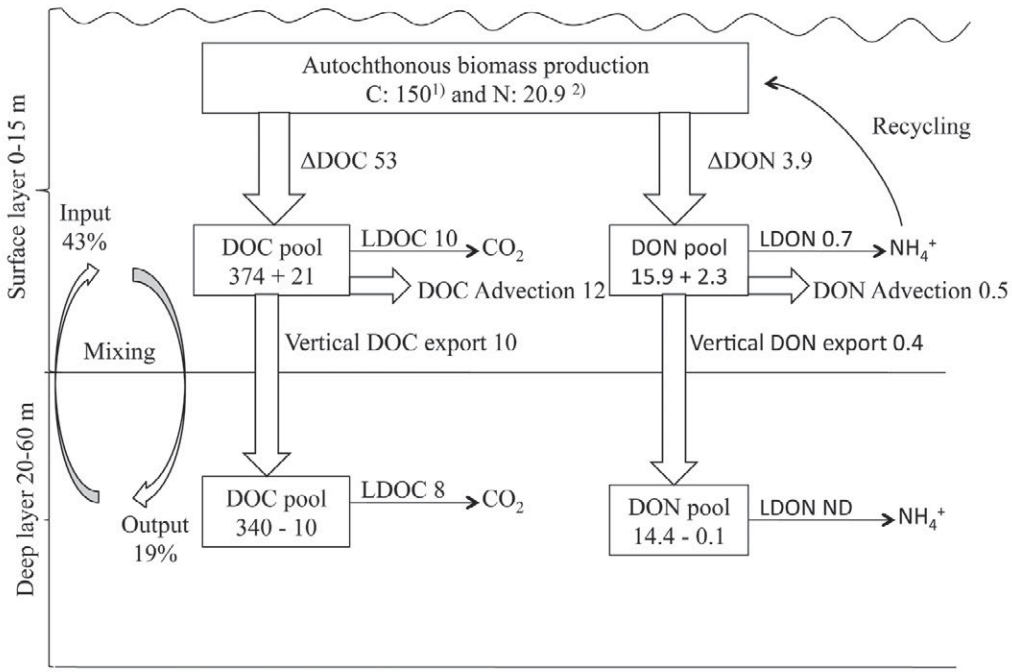


Fig. 4. Time course of phytoplankton growth in the W Gulf of Finland during the productive season in 2002. Vertical profiles of in vivo fluorescence in the archipelago (BEX1; A) and in the open-sea (LL11; B) sites and chl-*a* concentration in the surface water at 2.5-m depth on the shore-to-open-sea transect (C). Note different scales on x-axis. Figure redrawn from III.

Table 5. DOC concentrations and molar C:N, C:P and N:P ratios for DOM in open-sea (Längden- LL11) surface water in the Gulf of Finland during different stages of phytoplankton growth in 2002 (± SD). Table redrawn from III.

Stage	Date	DOC μmol C l^{-1}	C:N	C:P	N:P
Winter	14–16 Jan	361 ± 7	28.3 ± 1.8	ND	ND
Early spring	10 Apr	361 ± 6	25.9 ± 0.5	ND	ND
Spring bloom	24 Apr–7 May	385 ± 13	23.2 ± 1.0	1032 ± 176	51.8 ± 7.5
Summer minimum	22 May–3 Jul	388 ± 10	22.7 ± 1.6	837 ± 335	43.1 ± 3.7
Cyanobacterial bloom	17–31 Jul	398 ± 12	21.7 ± 1.4	664 ± 70	35.8 ± 3.7
Late summer - autumn	14 Aug–25 Sep	394 ± 16	21.5 ± 1.3	594 ± 74	32.2 ± 3.4

Fig. 5. Conceptual model of the dynamics of autochthonous DOC and DON pools (μmol l^{-1}) in the surface layer of the open-sea water of the W Gulf of Finland during the stratified 4-month summer period (May–August) in 2002. Autochthonous DOM is released via processes such as zooplankton "sloppy feeding" and autolysis of phytoplankton, and lost via vertical mixing over the thermocline, horizontal advection and bacterial degradation (LDOC and LDON, degradable within 2 weeks). Autochthonous production values (with Redfield C:N ratio) are based on recorded phytoplankton biomass and reported indigenous growth rate (0.6 d^{-1}; Lignell et al. 2003). The contemporary 4-month salt and water mass balance budget (III) gave 43 % dilution of surface layer by deep-water inflow, which was balanced by nearly equal shares of advection and vertical outflow. DOM pools at the beginning of the stratification period (early May) plus seasonal net changes by early September (preautumn overturn) are shown in boxes. ΔDOC and ΔDON values denote the estimated total DOM increase, accounting also for flux into deep water. Figure redrawn from III.



regression lines in element-element plots. The slopes obtained, described in detail in III, suggested that changes in the DOM pool occurred, with C:N:P ratios of 238:14:1.

The net flows of DOC and DON in the surface layer of the GoF during the 4-month-long summer stratification period (May–August) are summarized in a conceptual model (Fig. 5).The total accumulation of DOM during the summer stratification period was estimated by constructing a steady-state budget for water and salt mass flows between the key GoF basin compartments (III). The steady-state budget suggested a 43 % dilution of the surface layer volume by vertical deep-water inflow over the stratification period (Fig. 5). Adding the product of this turnover and the average extra DOC, DON and DOP in the surface layer, compared with deep water, to the net accumulation of these pools in the surface layer resulted in total accumulation values of $\Delta DOC = 53$ μmol C l^{-1}, $\Delta DON = 3.9$ μmol N l^{-1} and $\Delta DOP = 0.32$ μmol P l^{-1} (Fig. 5; III).

The extra DOC in the surface water compared with the deep-water value of 50 ± 10 mmol C m^{-3} in early autumn 2002 (9 September) was estimated to result in vertical export of DOC of approx. 560 mmol C m^{-2} from the 15-m surface layer into the 45-m deep-water layer during the autumn overturn (Fig. 5; III). The export of DON to deep water during the autumn overturn in 2002 was analogously approx. 34 mmol N m^{-2} and export of DOP 4.6 mmol P m^{-2} (Fig. 5). Taking into account the average extra DOC, DON and DOP in the surface layer compared with the deep water and the estimated downward flow of 19 % of the surface volume during the thermal stratification period (III), the estimates obtained for the total vertical export of DOC, DON and DOP from the surface layer to deep water during the stratification period and autumn overturn were 710 mmol C m^{-2}, 40 mmol N m^{-2} and 5 mmol P m^{-2} (III).

5.2. Accumulation of DON in a mesocosm experiment conducted during a cyanobacterial bloom (this thesis)

The accumulation of DON during a cyanobacterial bloom was also followed in a mesocosm experiment conducted in July 2003 to estimate the percentage of fixed N that is stored in the DON pool. In the control mesocosm, linear regression fitted

Table 6. Rates of DON accumulation (µmol N l^{-1} d^{-1}) in a mesocosm experiment conducted in a sheltered archipelago site near the Tvärminne Zoological Station in the western Gulf of Finland during a cyanobacterial bloom in July 2003. The rates were derived from the slopes of linear regressions of time courses of DON. Significance of the slopes was determined with T-statistics. Significant slopes are denoted with asterisks (* = $p < 0.1$, ** = $p < 0.05$). Accumulation of DON was related to cyanobacterial N_2 fixation values and accumulation of cyanobacterial biomass reported in the same mesocosm bags (Kangro et al. 2007), using a cyanobacterial C:N ratio of 6.2 (Larsson et al. 2001).

Mesocosm	Fitted period (d)	Intercept	Slope (d^{-1})	DF	DON increase: fixed N (%)	DON increase: Cyanobacterial N biomass increase
Control	0–22	17.20	0.21**	31	34	1.2
P	6–22	17.15	0.24**	23	72	1.0
5P	6–22	18.62	0.06	22	17	0.6
5PG	6–22	18.04	0.10*	23	31	0.8

to DON increase gave an accumulation rate of 0.21 μmol N l^{-1} d^{-1} (Table 6). For P-treated mesocosms (P, 5P, 5PG), the linear regression was fitted to the days (6–22) after an initial 5-day boosting period with combined N and P additions, giving DON accumulation rates of 0.06–0.24 μmol N l^{-1} d^{-1}. The cyanobacterial biomasses and N_2 fixation rates in these mesocosms are presented in Kangro et al. (2007).

5.3. Biological degradability of DOM (I, III, this thesis)

During the summer minimum period in 2001 and late summer cyanobacterial bloom in 2002 in the W GoF, the biological degradability of the open-sea surface DOC and DON were followed with three series of 2–3-week incubations of natural bacterial (< 0.7 μm) samples. Bacteria degraded the LDOC and LDON pools within 1 week (daily measurements). During the summer minimum period in 2001, the LDOC ranged from < 1 % to 3 % of the total DOC, according to linear regressions fitted to the 1-week declining period (Table 7). The DON pools were generally more labile than the DOC pools (Table 7). During the summer minimum period in 2001, linear regressions fitted to the 1-week declining period in different treatments gave LDON values ranging from 13 % to 21 % of the total DON in the W GoF (I).

Spatial and seasonal variation in the biological degradability of DOM was followed with 2–3-week end-point incubations of natural bacterial (< 0.7 μm) samples. The LDOC was determined from samples with NP treatment, whereas LDON was determined from the samples with combined P and glucose-C treatment (PC), except for experiments along the E-W transect in which only the NP treatment was conducted. In late July–early August 2001, the percentages of LDOC and LDON along the E-W transect were 2–4 % and 14 % of the respective total DOC and DON pools (Table 7). No clear E-W gradient in the biological degradability of DOM was found, showing that the labile pools were not site-specific (Table 7). In January 2002, no significant DOM degradation occurred during the 3-week incubations, even in the samples incubated at elevated temperatures.

During the spring, summer minimum period and cyanobacterial bloom in 2002, the LDOC pool ranged from 0 % to 4 % of the total DOC (III; Table 7), agreeing with the results from the 2001 experiments (I). During and after the decline in the late summer cyanobacterial bloom (14 August–25 September), the percentage of LDOC was higher ranging from 5 % to 9 % of the total DOC. During the spring bloom and early phase of the cyanobacterial bloom (mid-July), all LDOC was utilized within 2 days, whereas during late summer and autumn, 40–88 % of the LDOC pool was utilized within 2 days.

No significant nutrient or flagellate treatment effects on LDOC utilization were generally recorded (I, III). The only exceptions, when degradability was highest in samples with combined NH_4^+-N and PO_4^{3-}-P addition (data not shown), appeared to stem from increased LDOC availability coinciding with transport of LDOC by low-salinity water masses in mid-June 2002 and maximal LDOC availability (9 % of total DOC) during the decay of the cyanobacterial bloom in late August 2002 (Fig. 4C, Table 7).

The percentage of LDOC in open-sea deep water varied between 0 % and 9 % of the total DOC pool, following a pattern similar to that of surface samples (Table 7). During the spring bloom, the LDOC

Table 7. Labile DOC (LDOC) and DON (LDON) concentrations (μmol l^{-1}) and C:N ratios of labile DOM (LDOM) in the Gulf of Finland in 2001–2002. In experiments conducted during the summer minimum period in 2001 and on 17 July 2002, LDOC and LDON were estimated by fitting linear regressions to the approx. 1-week declining periods of total DOC and DON (daily measurements), and the significance was determined with T-statistics from the regression slopes (five replicates). In the other experiments, LDOC and LDON were determined from depletion of the pools in 2-week or 3-week (winter) end-point incubations and the significance of the depletion was tested with two sample T-tests (five replicates). For winter, surface samples from incubation T = 12 °C are presented. The percentage of LDOC and LDON in the respective total pools is given in brackets. For accumulation of DOM, mean values of all open-sea sampling sites (LL11-Längden) ± SD are given. 0 = no LDOM detected. Significant DOM depletion values are denoted with asterisks (* = $p < 0.1$, ** = $p < 0.05$). Sampling sites in Fig. 1. Table modified from I and III.

			Surface					Deep	
Period	Date	Site	LDOC ± SD	(%)	LDON ± SD	(%)	C:N of LDOM	LDOC ± SD	(%)
Summer minimum	14 Jun 2001	Lå	3.1	(0.8)	3.7$^{**1)}$	(20)	0.8	ND	
	4 Jul 2001	Lä	10.7**	(2.8)	2.5**	(14)	4.3	ND	
Bloom of cyanobacteria	30 Jul 2001	LL3A	10.5 ± 2.4**	(2.2)	3.0 ±1.4$^{**1)}$	(14)	3.4	ND	
	31 Jul 2001	XV1	21.4 ± 3.0**	(4.5)	3.0 ± 0.3$^{**1)}$	(14)	7.2	ND	
	1 Aug 2001	LL7	10.2 ± 5.5**	(2.4)	2.8 ± 0.3$^{**1)}$	(14)	3.7	ND	
Winter	14 Jan 2002	LL5	0		0		-	0	
	16 Jan 2002	LL9	2.9 ± 1.8	(0.8)	0.8 ± 1.4	(7)	3.6	3.8 ± 5.1	(1.1)
	16 Jan 2002	LL11	2.8 ± 2.4	(0.7)	0		-	5.0 ± 2.8*	(1.5)
Early spring	9 Apr 2002	LL11	5.2 ± 2.7**	(1.4)	6.5 ± 0.7**	(41)	0.8	4.4 ± 1.2**	(1.3)
Spring bloom	24 Apr 2002	LL11	8.6 ± 2.7*	(2.3)	0		-	14.6 ± 8.8	(4.3)
	7 May 2002	LL11	14.5 ± 5.7	(3.8)	0		-	26.3 ± 2.5*	(7.3)
Summer minimum	22 May 2002	LL11	0		1.0 ± 1.4	(6)	-	0	
	5 Jun 2002	LL11	0		0		-	0	
	17 Jun 2002	LL11	13.4 ± 5.2**	(3.2)	0		-	0	
	3 Jul 2002	Lå	0		0.9 ± 0.8	(5)	-	7.0 ± 5.8	(2.0)
Bloom of cyanobacteria	17 Jul 2002	LL11	21.6**	(5.3)	ND		ND	2.1 ± 3.5	(0.6)
	31 Jul 2002	LL11	13.3 ± 13.6	(3.5)	0		-	10.8 ± 2.6	(3.2)
Late summer - autumn	14 Aug 2002	LL11	19.4 ± 8.6**	(4.7)	2.0 ± 1.1**	(11)	9.6	10.3 ± 11.6*	(3.1)
	28 Aug 2002	LL11	37.5 ± 6.9**	(9.1)	3.2 ± 0.8**	(16)	11.7	13.7 ± 5.5**	(4.2)
	11 Sep 2002	LL11	27.8 ± 11.2**	(7.0)	2.5 ± 1.7**	(12)	10.9	0	
	25 Sep 2002	Lä	23.3 ± 3.8**	(6.3)	3.9 ± 0.8**	(20)	6.0	34.7 ± 1.2**	(9.6)

[1)] LDON determined from NP-treated samples

ranged from 4 % to 7 % of the total DOC, whereas during the summer minimum period no LDOC was found. During and after the cyanobacterial bloom, the percentage of LDOC ranged between 0 % and 10 %, and the highest value was obtained after the breakdown of the thermal stratification in late September. In Pojo Bay, the surface water LDOC pool accounted for 2–8 % of the total DOC, with the highest values in mid- and late summer (data not shown).

In early April 2002, the LDON accounted for 40 % of the total DON in the open-sea surface water (III, Table 7). Later in spring, and in contrast to the results from year 2001 (I), during the summer minimum period no LDON was found. From mid-July to late September, the LDON pool varied generally between 10 % and 20 % of the total pool (Table 7). The highest percentage of LDON was obtained after the breakdown of thermal stratification in late September.

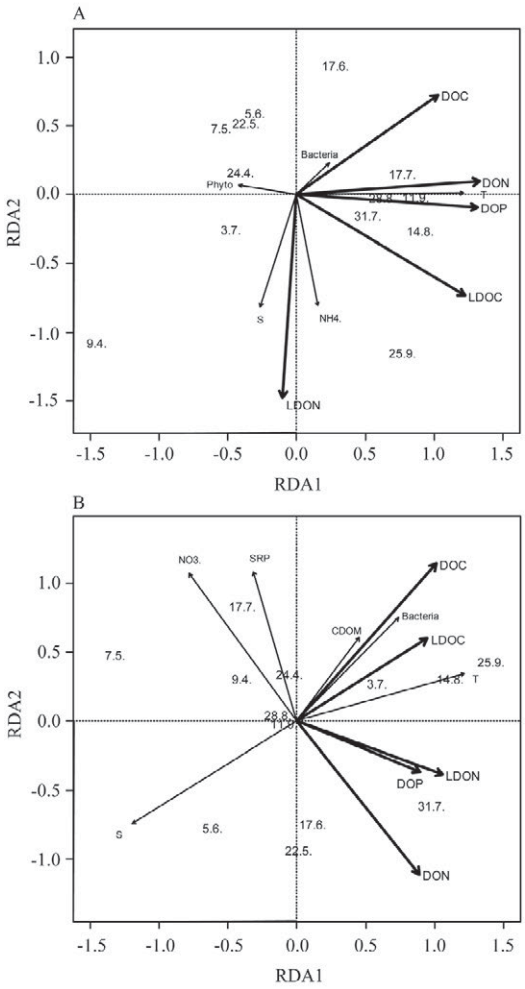
The PC treatment increased DON utilization significantly only in early April and from late August to September 2002 (data not shown).

The C:N ratios of LDOM were low, increasing from < 1 to 4 during the summer minimum period (NW GoF) to 3–7 during the cyanobacterial bloom in 2001 (E-W transect of the GoF; Table 7). In 2002, both LDOC and LDON were simultaneously recorded only after the cyanobacterial bloom, when the LDOC:LDON ratio ranged from 6 to 12.

5.4. Redundancy analysis of factors controlling the ambient DOM pools (III)

In 2002, the effects of key physical, chemical and biological factors on the net pools of different DOM constituents were evaluated with RDA for the open-sea surface and deep water in the W GoF. In the open-sea surface water, temperature and phytoplankton biomass explained most of the variation in concentrations of DOM constituents along the first RDA axis (RDA1; 61 % of total variation; Fig. 6A). The high positive correlation of temperature with most DOM constituents probably reflected the accumulation of DOM during the spring and summer phytoplankton succession (Figs 2, 3B, 6A). Accumulation of DOM compounds also occurred after the decline in the phytoplankton blooms and throughout the summer, which was reflected in negative correlations between the DOM constituents and total algal biomass. Salinity was important in explaining the DOM variation along the second RDA axis (RDA2; 18 % of total variation; Fig. 6A). The negative correlation between salinity and DOC reflected the DOC peak co-occurring with low-salinity water masses, which again probably were transported from the NW coast line of Finland by persisting, strong NW winds in mid-June (see 5.1; Figs. 2E, 3B, 6A).

Fig. 6. Redundancy analysis (RDA) of the correlation of different DOM constituents with physical, chemical and biological variables in the open-sea Gulf of Finland during the productive season in 2002. Correlation of A) surface and B) deep-water DOM with physical factors (S = salinity, T = temperature, FLRC = fluorescence, CDOM absorption), inorganic nutrients, and phytoplankton and bacteria along the first and second RDA axes. The dates show the distribution of separate samplings along the first two RDA axes. Figure redrawn from III.



In open-sea deep water (20–60 m), temperature and salinity explained most of the variation along the first RDA axis (RDA1; 41 % of total variation; Fig. 6B). Temperature in the deep water increased during the productive season, and the positive correlation between some of the DOM constituents and temperature probably reflected a downward flow of DOM from the surface to deep water via vertical mixing events during the stratified period (Figs. 2E, 3B, 6B). Deep-water salinity slightly decreased during the productive season, which probably contributed to its negative correlation with DOM constituents (Figs 2E, 3D, 6B). Most of the DOM variation along the second RDA axis (RDA2; 17 % of total variation) was explained by variation in the concentrations of soluble reactive P (SRP) and NO_3^-, which were negatively correlated with the DON, DOP and LDON concentrations, probably reflecting, at least in part, microbial DON and DOP remineralisation.

5.5. Factors controlling bacterial growth (I, II)

During the summer minimum period in 2001 and late summer cyanobacterial bloom in mid-July 2002, the effects of inorganic nutrient (N and P), glucose and HNF treatments on bacterial growth were followed with 2-week incubations of indigenous bacterial samples (I). The time courses of bacterial production and biomass suggested that the labile fractions of the indigenous DOM pools were used up within 1 week, coinciding with the declining phases of the DOC and DON pools. Both during the summer minimum period in early July 2001 and the cyanobacterial bloom in mid-July 2002, these bioassays suggested primarily C-limited bacterial biomass and production (I).

In 2003, the effects of inorganic nutrient (N and P) and glucose-C treatments on bacterial growth were followed for 3 days in natural surface and deep-water bacterial

Table 8. Important responses of bacterial production to nutrient (NH_4^+-N, PO_4^{3-}-P and glucose-C) and temperature (T) treatments in 3-d bacterial incubation experiments in W GoF in spring and summer 2003. Experiments followed factorial design with all combinations of treatments. Significant regression coefficients for treatment effects were extracted, using orthogonal regression analysis, and are given in the order of importance suggested by normal probability plot examinations. The values in the parenthesis represent the corresponding percentage increase in integral 3-d bacterial production response (vs. control). In deep-water samples, NPC effect showed only at surface water temperature. Missing values are denoted with dashes; # = only bottle effect important. The coefficient of determination (r^2) for the corresponding regression models (polynomial fit) ranged from 0.74 to 0.95. Table modified from II.

	Main treatment effects (r^2) [percentage change vs. control]			
Sample (treatments)	Spring 12 May	Minimum 9 June	Minimum 1 July	Cyanobacterial bloom 11 August
Surface (N, P, C)	NC (56 %)	C (86 %), NC (223 %)	C (144 %), N (69 %), NC (749 %)	C (67 %), NPC (554 %)
Deep (NP, C)	#	C (268 %)	-	C (322 %)
Deep (T, NPC)	T (200 %)	T (414 %), NPC (624 %)	-	T (606 %), NPC (173 %)

samples during the main postspring bloom stages of phytoplankton growth (II). Orthogonal regression analysis suggested that C was the primary limiting nutrient for bacterial production in surface water (Table 8). Glucose-C addition increased bacterial production significantly throughout the summer, but rapidly led to inorganic nutrient limitation. Addition of both glucose-C and inorganic nutrients further increased bacterial production significantly (II, Table 8). From May to July, when the DIN:SRP ratio (< 1–5) was below the Redfield ratio (Redfield 1958), combined N and glucose-C treatment significantly increased bacterial production. During the cyanobacterial bloom in August, when both the DIN and SRP were depleted in the surface water, inorganic N and P were both needed in combination with glucose-C for a significant combined bacterial production response to be seen (Tables 4, 8).

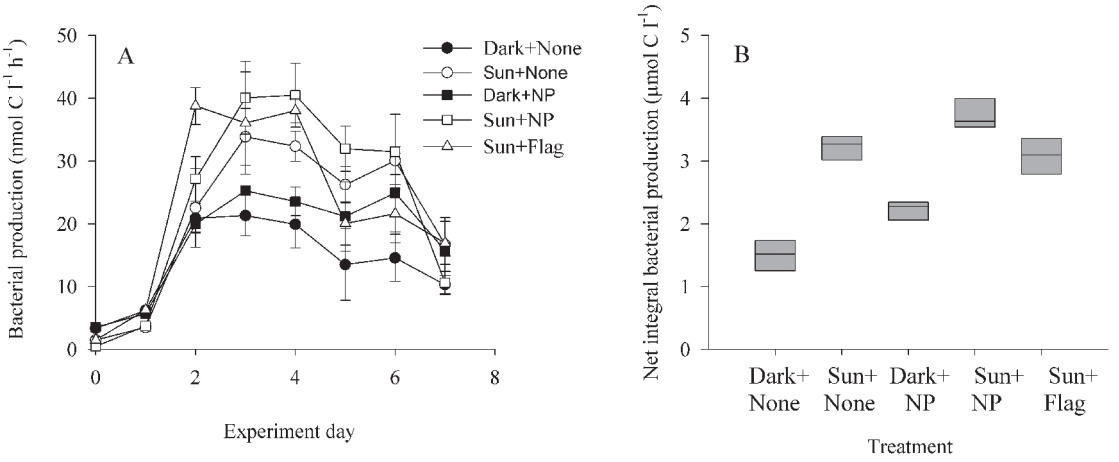
In deep-water samples, elevation of the incubation temperature led to significantly increased bacterial production and biomass in all experiments (II, Table 8). In May, bacterial production also remained low at an increased temperature (10 °C) and no nutrient treatment effects were found. In June and August, glucose-C treatment induced a significant increase in bacterial production, whereas NP treatment did not significantly affect bacterial production in any of the experiments.

5.6. Effects of photochemical transformation of DOM on microbial growth (I, II, IV)

5.6.1. One-day sunlight preexposure (I, II)

A 1-day sunlight preexposure of particle-free DOM samples (sunny day, *in situ*) generally stimulated bacterial production in subsequent incubations (I, II). In early July 2001, sunlight preexposure of DOM samples from the NW GoF was done at 20 cm depths leading to 69–115 % higher integral production responses over 1 week

Fig. 7. A) Effects of sunlight (vs. dark) preexposure of DOM samples on time courses of bacterial production and B) corresponding net integral production responses over the initial 1-week stimulation period at Längden on 4 July 2001. Sample treatments in Table 2. Error bars = SD (n = 5). Figure redrawn from I.



compared to the corresponding dark samples (Fig. 7; I). NP treatment of sunlight-exposed samples (Table 2) led to further significant increase in bacterial production. In late July 2001, integral bacterial production over 1 week in the NP-treated samples from the E-W transect of the GoF was, in turn, 14–38 % higher in the samples with sunlight pre-treatment than in the dark controls (I).

In 2003, the < 0.2-μm DOM samples from Pojo Bay and the outer archipelago and open-sea sites in the NW GoF were exposed to sunlight over 1 day at four depths (0.1–2 m) during the main postspring bloom stages of phytoplankton growth (II). Significant integral bacterial production responses were restricted to samples incubated at a depth of 10 cm. In the outer archipelago and open-sea samples, sunlight pre-treatment tended to have a positive effect on integral bacterial production both in July (9–23 %) and August (19–44 %), but a statistically significant integral production response (44 %) was found only in the open-sea samples in August (II). In samples from Pojo Bay, the effect of sunlight pre-treatment on integral bacterial production was 35–41 % (II).

No significant changes in DOC or DON concentrations occurred in any of the experiments in 2001 and 2003 (I, II). However, in 2003 sunlight exposure generally induced a decrease (3.7 % ± 2.5 % of the initial absorption) in CDOM abs_{375nm}, ranging from 0.00 to 0.10 m^{-1} in the archipelago and open-sea samples and from 0.22 to 0.27 m^{-1} in the Pojo Bay samples.

5.6.2. Two-week sunlight preexposure of refractory DOM (IV)

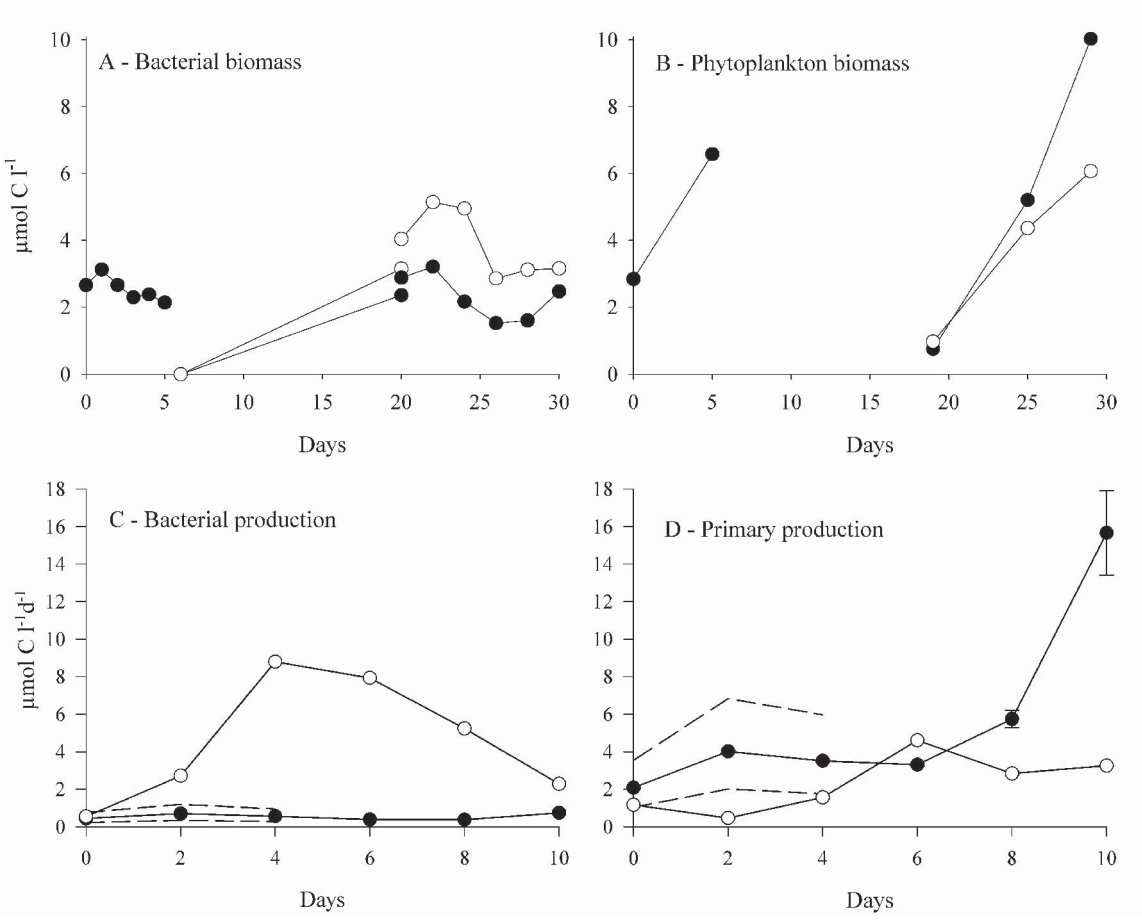
In early August 2005, after a 5-day pretreatment eliminated most of the ambient LDOC and LDON pools (IV), < 0.2-μm-filtered samples of refractory DOM were exposed to sunlight for 14 days (dark controls wrapped in aluminium foil). Bacterial growth occurred during the exposure, reaching higher biomasses in the sunlight-exposed samples than in the dark controls (Fig.8A).

After sunlight exposure, both the sunlight-exposed sample and the dark control were incubated with natural <10-μm inocula under PAR for 10 days. Based on thymidine incorporation, integral bacterial production over the entire 10-day bioassay was in the sunlight-exposed sample eight-fold higher than that in the dark control (time course of bacterial production in Fig. 8C; IV). Based on leucine incorporation, the difference was less distinct (two-fold), but nevertheless significant (IV). During the sunlight exposure and the subsequent bioassay, labile photoproducts supported bacterial production by 48 μmol C l^{-1} (thymidine incorporation) or 11 μmol C l^{-1} (leucine incorporation) (IV). These values were related to the number of photons absorbed during exposure to obtain the $\varphi_{bp,\lambda}$ for stimulated bacterial production (IV; Vähätalo et al. 2011). The rate of bacterial production based on photoproduced LDOM over the entire water column was derived as a product of the $\varphi_{bp,\lambda}$ obtained and the average dose of daily summer solar radiation, assuming that CDOM absorbed all the photolytic solar radiation (Vähätalo et al. 2011). The estimates obtained were 1.63 mmol C m^{-2} d^{-1} and 0.33 mmol C m^{-2} d^{-1}, based on thymidine and leucine incorporation, respectively (IV).

The bacterial biomass remained higher (68 % by average) in the sunlight-exposed water than in the dark control throughout the bioassay (Fig. 8A). The bacterial biomasses declined prior to the HNF peaks, indicating grazing of bacteria by HNF. The biomass peaks of the HNF and their ciliate grazers were higher by 50–76 % in the sunlight-exposed sample than in the dark control (IV).

Primary production in the dark controls was about three times as high as in the sunlight-exposed samples when integrated over the first 4 days of the bioassay (Fig. 8D). Similarly, the chl-*a* and accumulation of phytoplankton biomass were higher in the dark control than in the sunlight-exposed sample (Fig. 8B, Fig. 2D in IV). The DIN was depleted during the bioassay in both treatments, whereas the SRP concentration remained high (≥ 1.5 μmol P l^{-1}) in both treatments throughout the experiment, indicating N-limitation of phytoplankton growth.

Fig. 8. Effects of photochemical transformation of refractory DOM on bacterial and algal growth at Långskär in July–August 2005. The biomasses of (A) heterotrophic bacteria and (B) phytoplankton during the three phases of the experiment (days 0–6: pretreatment to remove DIN and labile DOM, days 6–19: sunlight exposure of refractory DOM, and days 19–29: bioassay with < 10-μm plankton assemblage), and C) bacterial production and D) primary production during the bioassay in the sunlight-preexposed samples (open circles) and their dark controls (filled circles). The dashed lines in (C, D) show the 95 % confidence limits evaluated for the initial 0–4-d response in the dark control (type B uncertainty, IV). The error bars in (D) show the range in primary production in two replicate incubation bottles. Figure redrawn from IV.



5.7. Effects of photochemical transformation of DOM on bacterial community composition (IV)

A clone library was made of the indigenous plankton inoculum used in the bioassay of the sunlight exposure experiment in late summer 2005. Of the 96 clones sequenced, 82 were affiliated with bacteria that belonged to 35 different operational taxonomic units (OTUs), using 97 % sequence similarity in delineation. According to the clone library, most clones were affiliated with Actinobacteria, α-Proteobacteria, Cyanobacteria and Bacteroidetes (Fig. 9). Individual clones were obtained from the *γ*-Proteobacteria and the order Burkholderiales of *β*-Proteobacteria. Most actinobacterial clones clustered within the orders Acidimicrobiales and Actinomycetales, or within the freshwater bacterium Candidatus *Planktophila* (94–97 % similarity) of the acI cluster. Most Bacteroidetes clustered within the order Flavobacteriales. Almost all *α*-Proteobacteria clustered within Candidatus *Pelagibacter* of the SAR11clade (87–100 % similarity). The *γ*-Proteobacteria were affiliated with SAR86 cluster (93–100 % similarity).

Fig. 9. Relative percentages of bacterial phyla and classes of Bacteroidetes and Proteobacteria in the clone library of a surface water sample (< 10 *μ*m) from Långskär on 31 July 2005. The sample was distributed as inocula to the sunlight-exposed DOM sample and dark control at the beginning of the bioassay in the experiment on the effects of photochemical transformation of refractory DOM on microbial growth, conducted in July–August. Affiliation of the clones with bacterial genera in the Ribosomal Database is shown with similarity in parentheses. Figure redrawn from IV.

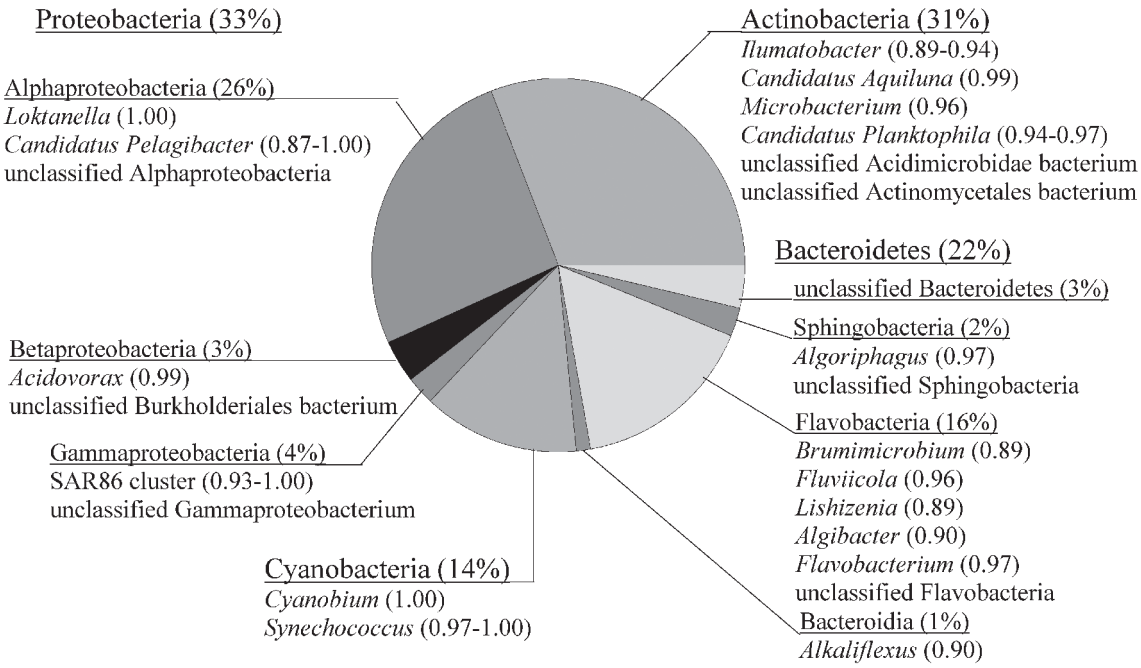
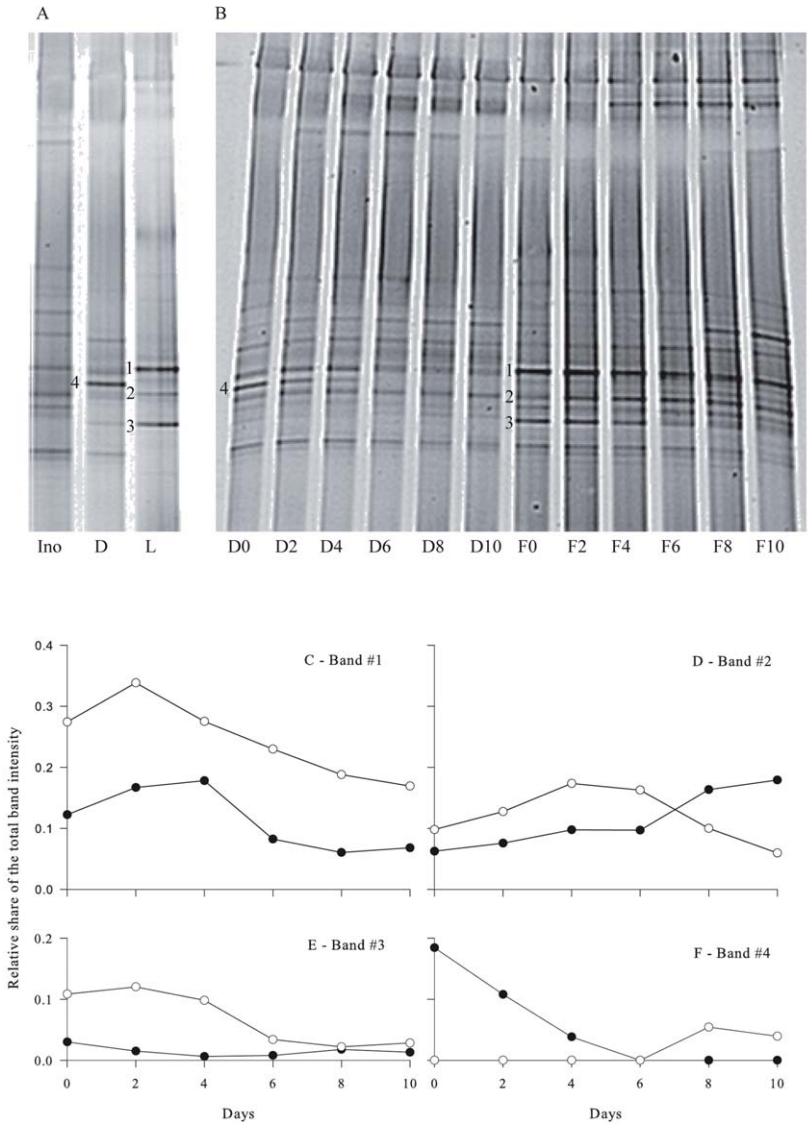


Fig. 10. Changes in the composition of the surface water bacterial community at Långskär in the experiment on the effects of photochemical transformation of refractory DOM on microbial growth, conducted in July–August 2005. Bacterial community composition profiles were analysed with DGGE (A) in the sunlight-exposed sample and the dark control at the end of the sunlight pretreatment and in the < 10-μm inoculum (Ino) added to the samples for the bioassay as well as (B) in the sunlight-exposed sample and the dark control during the bioassay. (C–F) Time courses during the bioassay of the relative DGGE band intensities of the bacterial OTUs affected by the photochemical transformation of DOM. Affiliation of the sequenced bands in the Ribosomal Database (similarity): 1) *Polynucleobacter necessarius* (99 %), 2) *Acidovorax facilis* (100 %), 3) *Hydrogenophaga* sp.(100 %) and 4) uncultured bacterium of γ-Proteobacteria (99 %; Table 2 in IV). Figure redrawn from IV



Almost all Cyanobacteria were affiliated with *Synechococcus*. DGGE analysis implied a higher percentage of *β*-Proteobacteria in the bacterial community than the clone library, the *β*-proteobacterial bands sequenced comprising 16 % of the total band intensity in the inoculum sample.

DGGE analysis was applied for an insight into the effects of the sunlight exposure on the composition of the bacterial community during the sunlight exposure and the subsequent bioassay. In all, 23 different bacterial bands were detected from the DGGE gel (Fig. 10A). The number of bacterial bands in the DGGE gel was 21 and 12 in the inoculum and the sunlight-exposed sample, respectively. The band patterns revealed that the bacterial community in the exposed water differed from that in the dark control at the end of sunlight exposure. The sunlight exposure of DOM benefited most clearly three bacterial OTUs (bands 1–3), all of them affiliated with *β*-Proteobacteria (Fig. 10A, Table 2 in IV). These *β*-proteobacterial bands clustered within the genus *Polynucleobacter* (99 % similarity, band 1) of the family Burkholderiaceae and with genera *Hydrogenophaga* (99 % similarity, band 2) and *Acidovorax* (100 % similarity, band 3) of the family Comamonadaceae, all belonging to the order Burkholderiales.

Based on the relative band intensities of the DGGE gel, the bacterial OTUs (bands 1–3) that had most benefited from the photochemical DOM breakdown during the sunlight exposure increased their relative intensity in the bacterial community of the sunlight-exposed water during the first 2–4 days of the bioassay (Fig. 10). On day 2, these bands comprised together 59 % and 26 % of the total band intensity in the sunlight-exposed and the dark control samples, respectively. Based on the NMDS analysis of the relative band patterns, the dissimilarity between the treatments was highest during the first 2 days of the bioassay (Fig. 9 in IV). Towards the end of the bioassay, the dissimilarities between the bacterial communities in the sunlight-exposed water and the dark control became less distinct.

6. DISCUSSION

6.1. Seasonal variability in the DOM concentrations

DOM accumulated in the surface water of the GoF during the productive season (III), as occurs in several coastal and oceanic areas (e.g. Copin-Montégut & Avril 1993, Carlson et al. 1994, Williams 1995, Lønborg et al. 2009). Surface water accumulation began after formation of the thermocline by late April. The DOM concentrations varied horizontally, but due to an approx. 1-year water residence time in our study area (Andrejev et al. 2004), the DOM accumulation values were assumed to be locally representative. In support, similar DOM accumulation occurred throughout the archipelago-to-open-sea salinity gradient.

Dilution of the accumulated DOM out of the surface layer was taken into account with a steady-state budget for water and salt mass flows between the key GoF basin compartments (III). The corrected estimate of total seasonal DOC accumulation of 53 μmol C l^{-1} (Fig. 5) was within the lower end of the range of seasonal DOC accumulation values recorded for various marine areas (12–600 μmol C l^{-1}; e.g. Copin-Montégut & Avril 1993, Williams 1995, Zweifel 1999, Carlson et al. 2002). Similar values for DOC accumulation have been recorded elsewhere, e.g. in the coastal areas of the Gulf of Trieste, the English Channel, the coastal Pacific, and the Bothnian Sea (37–89 μmol C l-1;

Williams 1995, Zweifel et al. 1995, De Vittor et al. 2008).

6.1.1. Accumulation of autochthonous DOM

In coastal areas, the DOM accumulating in the surface water could have originated from autochthonous processes, inputs from allochthonous sources or both (e.g. Zweifel et al. 1995, Hopkinson et al. 2002). During the productive season in 2002, the profound increases in DOC and DON concentrations coincided with increases in phytoplankton biomass and *in vivo* fluorescence, indicating an autochthonous origin of the accumulating DOM. During the spring bloom, dominated by diatoms and dinoflagellates, the net DOC increase nearly equalled the increase in phytoplankton biomass. The highest DOC and DON concentrations occurred during and after the late summer bloom of diazotrophic cyanobacteria. However, RDA of the entire productive season showed a negative correlation between the DOM constituents and phytoplankton biomass, reflecting a situation in which the release and accumulation of DOM continued during the decay of the blooms. Thus, accumulation of DOM occurred both during actively growing phytoplankton blooms and during the decay of the blooms, conforming to results from the enclosed plankton communities (e.g. Norrman et al. 1995, Søndergaard et al. 2000).

Accumulation of DOM continued throughout the summer minimum period, with `sloppy feeding´ by microzooplankton as a plausible dominant supply pathway (cf. Kuosa 1991, Nagata 2000). During the spring bloom, the importance of grazing by zooplankton is small, due to late development of mesozooplankton (Kuparinen et al. 1984, Lignell et al. 1993), and the major bloom-forming cyanobacteria are poorly consumed by mesozooplankton (e.g. Sellner et al. 1994). The autochthonous DOM supply during these blooms was, hence, probably more dependent on release processes other than "sloppy feeding" by zooplankton, such as autolysis (death), viral lysis and direct extracellular release by phytoplankton (cf. Lignell 1990, Nagata 2000).

In the Baltic Sea, the amount of N_2 fixed by cyanobacteria is comparable to the riverine N load and exceeds the atmospheric N input (Larsson et al. 2001, Schneider et al. 2009). However, the fate of the fixed N_2 in the pelagic food web and its role in nutrient cycling remain largely unknown. The accumulation of cyanobacterial N biomass accounts for only a minor percentage of the N_2 fixation in the Baltic Sea, indicating large leakage of fixed N (Larsson et al. 2001, Rolff et al. 2007). In tropical oceanic waters an average of 50 % (range 0–76 %) of the newly fixed N is released into the water as DON by actively growing filamentous cyanobacterium, *Trichodesmium* (Glibert & Bronk 1994).

The percentage of fixed N that ends up in the DON pool in the GoF was approximated, using three approaches. Firstly, the total DON increase from early June to late July 2002 was related to the average cyanobacterial biomass during the same period and cyanobacterial C-biomass-specific N_2 fixation rates calculated from published Baltic Sea data (III) (Larsson et al. 2001, Kangro et al. 2007). The percentages obtained for DON ranged from 14 % to 100 % of the fixed N. Secondly, the amount of N_2 fixation was estimated from the total N increase in the surface layer during the same period, corrected for atmospheric DIN input and sedimentation losses (cf. Larsson et al. 2001). By this approach, the DON accumulation was estimated to account for 23–30 % of the N_2 fixation (III). Thirdly, accumulation of

DON in a mesocosm experiment conducted in the NW GoF in July 2003 was calculated to account for 39 % ± 24 % (mean ± SD) of the N_2 fixation by actively growing cyanobacteria in the same bags (N_2 fixation estimated in Kangro et al. 2007) (Table 6). The accumulation of DON was comparable to the increase in cyanobacterial N biomass in the respective mesocosm bags (Kangro et al. 2007), using a cyanobacterial C:N ratio of 6.2 (Larsson et al. 2001). Together, these estimates show that the DON pool acts as an important temporal storage for fixed N in the Baltic Sea, greatly exceeding the percentage of fixed N that is subsequently transferred to picoplankton from actively growing cyanobacterial communities in the Baltic Proper (5–10 %; Ohlendieck et al. 2000).

In the Baltic Sea, the high bulk DOC concentrations are driven by large inputs of terrestrial DOC that comprises over 60 % of the ambient total DOC pool (Alling et al. 2008). The effects of allochthonous DOM inputs on seasonal variability in the net DOM pools of open-sea water appeared, however, to be local and short-lived, as suggested by the short-lived appearance of water masses of lower salinity. In contrast to the results of this thesis for the GoF, riverine inputs dominated the seasonal accumulation of DOC in the Bothnian Sea, another northern basin of the Baltic Sea (Zweifel et al. 1995). This difference is also reflected in the higher BCD in relation to primary production in the Bothnian Sea (1–3) compared to the GoF and the Baltic Proper (0.5–0.7) (Hagström et al. 2001). The total accumulation of DOC corresponded to approx. 30 % of contemporary autochthonous production (Fig. 5), obtained from recorded phytoplankton biomasses, using a previously reported indigenous growth rate (0.6 d^{-1}; Lignell et al. 2003), and to approx. 15 % of the integral primary production previously reported for the thermally stratified period in the GoF (Lignell 1990), whereas the DOC accumulation equalled that of the fixed C in the Bothnian Sea (Zweifel et al. 1995).

6.1.2. Stoichiometry of DOM

The DOM pool in the surface water of the open-sea GoF had higher C:N and N:P ratios (annual average of C:N:P ratios of 794:41:1) than the average values reported for temperate and tropical oceanic surface waters (300: 20:1; Benner 2002) and temperate and subtropical oceanic surface waters (average 374: 27:1; Hopkinson & Vallino 2005). Even more extreme C:N:P ratios for surface water DOM (2460:100:1) have been found in the Bothnian Sea (Zweifel et al. 1995), whereas the C:N ratios in two coastal sites of Denmark, southern Baltic Sea were clearly lower (11; Lønborg & Søndergaard 2009). The high bulk DOC concentrations in the Baltic Sea are driven by terrestrial DOC inputs (Alling et al. 2008), and riverine inputs to the GoF with high C:N:P ratios of approx. 2500: 100:1 (Stepanauskas et al. 2002) clearly contribute to the high C:N:P ratios of the DOM pool.

During the 4-month spring-summer period with temperature stratification (May–August), the total DOM accumulation (dilution included) occurred with a C:N:P ratio of 165:12:1, i.e. clearly lower C:N, C:P and N:P ratios than those of the bulk DOM pool. A C:N ratio of total accumulated DOM of 14 (Fig. 5) was within the range occurring in marine surface waters (6–20; Williams 1995, Carlson et al. 2000). Marine mesocosm experiments have shown C:N and C:P ratios of newly produced DOM from 11 to infinite (no DON production) and from 17 to 500, respectively, often with notable increasing impacts of nutrient limitation (Norrman et al. 1995, Søndergaard et al. 2000, Conan et al.

2007). The clearly lower C:N, C:P and N:P ratios of the accumulating DOM than those of the background DOM and decreases in these ratios in the total DOM pool throughout the summer suggest that the recycling efficiency of the DOM elements increased in the order C < N < P, as occurs in oceanic areas (e.g. Hopkinson & Vallino 2005).

The stoichiometry of the temporal, vertical and horizontal variations in the DOM pools across the transect from archipelago to open-sea was determined from the slopes of the linear regression lines in element-element (DOC vs. DON vs. DOP) plots, showing that these changes occurred on average with C:N:P ratios of 240:14:1, being close to the stoichiometry of the seasonally accumulating DOM in our study area, and also the stoichiometry of DOM production and decomposition found in oceanic areas (199:20:1; Hopkinson & Vallino 2005). Thus, despite the large background pool of allochthonous DOM with high C:N:P ratios in the GoF, the autochthonous DOM appears to dominate the variability in the DOM pools within a timescale of ≤ 1 year.

6.1.3. Comparison with previous DOM data from the Baltic Sea

The concentrations of DOC and DON in 2001–2005 suggest low interannual variability in these pools. Previous information on DOM concentrations in the GoF is scarce. In 1979, the concentrations of DOC in the surface layer at the Längden site in the W GoF were mostly within the range found in this study (Leppänen & Tamelander 1981). However, the variation with time in their DOC values, estimated from the difference of total organic C and particulate organic C (POC) concentrations, was larger. The average DOC concentration of biweekly measurements from April to September was 10 % lower in 1979 than in 2002, possibly reflecting the varying methodology between the studies. Nevertheless, it cannot be ruled out that the level of DOC has increased concomitantly with the increase in trophic state of the W GoF within this period (e.g. Raateoja et al. 2005). Previous estimates for the concentrations of DON (total N-(PON+DIN)) and DOP (total P- (POP+DIP)) in the surface water of the GoF for the productive season in 1979 (Leppänen & Tamelander 1981) and for late summer in 1990 (Kononen 1992) showed clearly larger variation with time than the range obtained in 2002 (III), probably reflecting in part the more dynamic nature of the DON and DOP pools than the DOC pool.

In the open-sea surface water of the GoF, the concentrations of DOC in May–June 2002 (393 ± 10 μmol C l^{-1}; III) were clearly above the values (300-325 μ mol C l^{-1}) recorded in the surface water across the Baltic Sea from the Bothnian Bay in the north to the Kattegat in the south for the same period in 1996 (Wedborg et al. 1994). In the Gulf of Riga, Baltic Sea, the concentrations of DOC were even higher, ranging from 420 to 1230 μmol C l^{-1} (Zweifel 1999). The high DOC concentrations in the Gulf of Riga were explained by river discharge (Zweifel 1999). In the GoF, a clear E-W gradient in the concentrations of DOC and DON (study III) suggests that the concentrations were affected by discharge to the eastern part of the GoF, mainly from river Neva, as is the case for the concentrations of total nutrients, POM and chl-*a* (Pitkänen et al. 1993, Perttilä et al. 1995, Kuuppo et al. 2006).

6.2. Biological DOM availability and factors controlling bacterial growth

6.2.1. Surface layer

Bioassays done during the main postspring bloom stages of the phytoplankton growth in 2003 showed that bacterial production was primarily limited by LDOC availability in the surface water of the GoF (II), as it was in summer 2001 (I). During most of the summer in 2003, combined addition of NH_4^+-N and glucose-C led to significant increases in bacterial production, showing deficiency of both LDOC and N. Low inorganic N:P ratios during the summer minimum periods in 2001–2003 implied that the plankton food web was N-limited, conforming to several previous studies (e.g. Kivi et al. 1993, Lignell et al. 2003). Thus, it appears that phytoplankton in the GoF exhaust the available DIN pools, while the bacterial assemblage is consistently limited by low availability of LDOC. This creates a situation in which both C and N are in short supply for bacterial growth (I, II).

Previous enclosure studies conducted in the GoF have shown positive bacterial production responses to glucose-C and inorganic nutrients (Lignell et al. 1992, Kuparinen & Heinänen 1993). Stimulation of bacterial production by inorganic nutrients has previously occurred during the spring bloom and after the late summer bloom of cyanobacteria (Kuparinen & Heinänen 1993), probably reflecting ample availability of autochthonous LDOC during these periods. Occasional inorganic nutrient limitation of bacterial production has also been encountered in mesocosm experiments, in which the plankton community processes such as zooplankton "sloppy feeding" and algal exudation provide a continuous supply of DOM (Kivi et al. 1993, Lignell et al. 2003).

The concentrations of LDOC, degradable within 1–2 weeks, were low from April to late July in summers 2001 and 2002, ranging from 0 to 22 μmol C l^{-1} (0–5 % of the total DOC). In a review of studies from various aquatic environments, an average of 19 % of the marine DOC was biologically labile, while the concentrations of LDOC, degradable within 1–2 weeks, ranged from 18 to 110 μmol C l^{-1} (Søndergaard & Middelboe 1995). A similar degradability range, from 17 % to 30 % of the total DOC, was again summarized by Hopkinson et al. (2002) for various marine environments. Concentrations of LDOC comparable to those in the GoF have been encountered in the pristine York River estuary, in the surface waters of the Sargasso Sea and in a mesocosm experiment with marine plankton communities (0–25 μmol C l^{-1}; Raymond & Bauer 2000, Søndergaard et al. 2000, Carlson et al. 2002). The size of the ambient (net) pool of LDOC is controlled by the balance between release and loss processes. Accordingly, the low LDOC concentrations recorded in the GoF evidently reflected efficient utilization of LDOC by the predominantly C-limited bacterial assemblage.

The concentrations of LDON were more variable than those of LDOC. During the summer minimum period and the bloom of filamentous cyanobacteria in 2001, LDON accounted for 14–20 % of the total DON, while during the spring bloom and summer minimum period in 2002, no LDON was found. The phytoplankton biomasses during the summer minimum period were also higher in 2001 than in 2002, probably leading to higher release of autochthonous DOM. The LDOC:LDON ratios recorded were low, ranging from < 1 to 7. The intracellular C:N ratio of bacteria is about 5 (Fagerbakke et al. 1996), and about 60 % of the LDOC taken up by bacteria was lost via respiration in

our study area (II). Thus, the low ambient LDOC:LDON ratios supported the view of C-limited bacterial growth, emerging from bacterial bioassay experiments.

During the blooms of filamentous cyanobacteria from mid-July to August 2001–2003, both inorganic N and P were depleted from the surface layer. In the August 2003 factorial experiment, the combined addition of both inorganic nutrients (N and P) and glucose-C induced a significant increase in bacterial production compared with the glucose-C-treated samples. The switch in secondary limiting nutrients of bacteria (with C as the primary limiting nutrient) from N to combined N and P availability by August clearly reflected the contemporary shift in the algal assemblage from distinct N limitation towards combined N and P deficiency, due to the N_2-fixing capacity of the predominant cyanobacteria (cf. Lignell et al. 2003). During and after the decline of the bloom, the concentrations of LDOC and LDON, ranging from 19 to 38 μmol C l^{-1} (5–9 % of the total DOC) and 2–4 μmol N l^{-1} (11–20 % of the total DON), indicated enhanced release of labile bacterial substrates. Accumulation of LDOC was probably enabled by the combination of strict grazing pressure on bacterial biomass by HNF and intensive competition for mineral nutrients between heterotrophic bacteria and phytoplankton, preventing enhancement of bacterial LDOM uptake along with enhanced LDOM release (cf. Thingstad et al. 1997, Thingstad & Lignell 1997). LDOM was in this study defined as DOM utilisable for the bacterial assemblage within 2 weeks (on average 60 % of LDOC was utilised within 2 days), and the degradability of LDOM for the bacterial assemblage apparently did not match the LDOM release rates (cf. Thingstad & Lignell 1997). Moreover, viral lysis of bacteria could have decreased the C transfer efficiency of the microbial loop, rereleasing DOM into the water (Suttle 2007).

6.2.2. Deep water

Temperature clearly limited the rate of bacterial production in deep water, supporting previous results from the same area (Autio 1990). Elevating the incubation temperature from the *in situ* value (about +3 °C) to near-surface water temperature (+10–16 °C) caused significant increase in bacterial production in late spring and during summer 2003. LDOC was also the growth-limiting substrate for bacterial growth in deep water, since glucose-C addition induced significant increases in bacterial production in samples incubated at elevated temperature, whereas combined addition of inorganic N and P did not markedly affect bacterial production, reflecting again ambient N- and P-replete conditions.

In accordance with the C limitation of bacterial growth, generally no LDOC was found in deep water during the summer minimum period in 2002. During the spring bloom, the concentration of LDOC, however, reached 7 % of the total DOC, probably reflecting decay of sedimenting diatoms and loss of dinoflagellate biomass from the surface layer via formation of cysts and slowly sinking phytodetrital material during the decline of the bloom (Heiskanen & Kononen 1994). A more persistent but moderate LDOC increase after the late summer cyanobacterial bloom, reaching 4 % of the total DOC, again probably resulted from the low settling rates of filamentous cyanobacteria and decomposition of most of the biomass in the water column (Heiskanen & Kononen 1994). The contribution of LDOC supply from the surface to the deep layers through surface layer turnover, e.g. via sporadic wind mixing, appeared to be minimal. Together, these results showed a similar situation

that prevails in the perennially cold Arctic Ocean, where temperature consistently limits bacterial growth, but within this boundary, the availability of LDOM determines bacterial production and growth rates (Kirchman et al. 2005).

6.3. Seasonal DOM export from the surface water

The percentage of total DOM, which is not degraded in the surface layer of the GoF by biological and photochemical processes, is susceptible to horizontal advection to the Baltic proper or to export to deep water via vertical mixing events and convection during the autumn cooling period. Relating the estimated total vertical export of DOM (III; 560 mmol C m^{-2}, 40 mmol N m^{-2} and 5 mmol P m^{-2}; Fig. 5) to published estimates of the sedimentation of POM suggests that DOC export in the GoF accounts for 12–25 % of the annual POC export (Elmgren 1984, Lignell 1990, Heiskanen et al. 1998) and DON and DOP exports 11 % and 20 % of the PON and POP exports, respectively (POP export was estimated from POC export using average of reported C:P ratios of POM export (123); Heiskanen et al. 1998). These percentages are low, compared with estimates ranging from 40 % to clearly above the POM flux found for various marine areas, including the Sargasso Sea, the Labrador Sea, the Mediterranean Sea and the subtropical Pacific Ocean (Copin-Montégut & Avril 1993, Carlson et al. 1994, Emerson et al. 1997, Avril 2002, Tian et al. 2004), but within the range estimated for the North Atlantic and the equatorial Pacific Ocean (9–30 %; Thomas et al. 1995, Carlson et al. 2010).

The extent of the DOM export and the quality of the compounds exported are dependent on the hydrodynamics and processes in the plankton food web. In oceanic areas, the intensity of the mixing markedly contributes to the magnitude of the DOC flux (Carlson et al. 1994, 2010, Tian et al. 2004). In the GoF, the relatively low contribution of DOC to total annual C export can partially be explained by rapid sedimentation of the main diatom-dominated spring bloom (cf. Heiskanen & Kononen 1994). Moreover, during most of the season of DOM accumulation, the primarily C-limited bacteria were nearly able to deplete the pool of LDOC, hence diminishing the C export, as was suggested to occur in the nutrient-replete Ross Sea, Antarctica (Carlson et al. 2000). Nevertheless, the vertical export of DOC appeared to form a notable temporal export term for C fixed by phytoplankton in the surface water of the GoF, accounting for 30 % of the estimated contemporary autochthonous biomass production (Fig. 5) and 9–13 % of published annual primary production values (Lignell 1990 and references therein). Since the availability of LDOC limits the growth of deep-water bacteria in the GoF, the export of labile and semilabile C from the surface to deep water represents a potentially important substrate supply for deep-water bacteria.

The export of DOM out of the surface layer occurred with clearly higher C:N and slightly higher C:P ratios (18 and 142, respectively) than the corresponding average ratios of settled particulate matter of 10 and 123 recorded previously in our study area for the same 4-mo period (May- August; Heiskanen et al. 1998), showing that C could be exported more efficiently out of the surface layer per given amount of nutrients via DOM than via sedimentation of POM. The same occurs in oceanic environments (Tian et al. 2004, Hopkinson & Vallino 2005). The N:P ratio of estimated vertical DOM export in the GoF was again low (8),

probably reflecting N limitation of the surface water plankton assemblage (e.g. Lignell et al. 2003), leading to preferred utilization of labile N compounds.

Horizontal advection to adjacent areas presents another marked loss term for accumulated DOM in coastal areas (Lønborg et al. 2009, Lønborg & Søndergaard 2009). The results of this thesis suggest that during the productive season, the DOM outflow to the Baltic proper was enriched with N and P, due to autochthonous processes in the open-sea GoF. However, the approx. 1-year water residence time in our study area (Andrejev et al. 2004) probably allows utilization of most of the accumulated LDOM and part of the semi-LDOM in the GoF, contrasting with findings of notable export of LDOM to adjacent areas from estuarine and coastal areas, with flushing times between 0.5–1 month (Raymond & Bauer 2000, Lønborg et al. 2009).

6.4. Responses of bacterial growth to photochemical DOM transformations

Bacterial production generally responded positively to sunlight preexposure of DOM, conforming to previous studies from various aquatic environments (reviewed in Moran & Zepp 1997, Vähätalo 2009). The effects of 1-day sunlight exposure of ambient DOM on bacterial production in subsequent 5-day to 2-week incubations with natural bacterial inocula varied from no effect to 115 % increase in production in summers 2001 and 2003. The bacterial responses in these experiments reflected the balance between the release of substrates labile for bacteria from initially refractory DOM (e.g. Moran & Zepp 1997, Vähätalo & Zepp 2005) and the transformation of initially biologically labile DOM, e.g. into CO_2, CO and more refractory DOM compounds (Benner & Biddanda 1998, Obernosterer et al. 2001, Vähätalo & Zepp 2005), explaining in part the wide variation in the bacterial responses. In the Gulf of Riga, where the availability of inorganic nutrients and LDOC is high during summer (Zweifel 1999), a series of 1-day sunlight exposures of DOM led to bacterial biomass responses varying from negative to positive (Jørgensen et al. 1999).

The effects of photochemical transformation of DOM on bacterial growth could be expected to decrease from the river mouth to the open sea, along with the steeply declining concentration gradient of CDOM, which absorbs > 90 % of the solar radiation that penetrates to the Baltic Sea (Babin et al. 2003), thus being susceptible to photochemical degradation. However, no consistent differences in the bacterial responses among the sites were found. Probably, the positive effect of labile photoproducts was masked by the initially high biological availability of DOC in the nutrient-rich Pojo Bay, where the concentrations of LDOC were in summer 2002 consistently high (37 ± 14 μmol C l^{-1}).

Significant effects of DOM photodegradation on daily bacterial production were limited to the top 0.2 m of the water column in our study area (II). Taking attenuation of photochemical reactions in the water column of the GoF into account (cf. Vähätalo & Zepp 2005), the increase observed in bacterial production at 0.1–0.2 m depths corresponds to an average increase in daily bacterial production by 0–7 % in the 10-m-deep mixed surface layer in the GoF (I, II).

In the experiment conducted in July–August 2005 (IV) and in our other similar experiment conducted in June 2005 (Vähätalo et al. 2011), exposure of refractory DOM to sunlight for 2 weeks led to notable

increases in bacterial production and biomass both during the sunlight treatment and the subsequent incubations with a 10 % inoculum of the natural <10-μm plankton community. The bacterial production supported by the photoproduced LDOM was then estimated, using $\varphi_{bp,\lambda}$ values and average daily solar doses (IV; Vähätalo et al. 2011). These estimates accounted for 2–9 % of the mean daily bacterial production in the surface water of the GoF during the summer minimum period and late summer bloom of filamentous cyanobacteria (IV). Together, these values were in the range of estimates based on 1-day sunlight exposure experiments, implying that during summer the labile photoproducts contribute < 10 % of the bacterial production in the surface water of the GoF.

The studies conducted in June (Vähätalo et al. 2011) and in mid-July–August both showed that part of the labile photoproducts assimilated by bacteria was transferred to higher trophic levels, via bacterivorous flagellates to ciliates. Since the labile photoproducts are mainly released from terrestrial DOM (Stedmon et al. 2007, Vähätalo et al. 2011), the photochemical transformations form a link between terrestrial DOM and planktonic production in the GoF (cf. Vähätalo et al. 2011). However, the quantitative importance of this link seems to be small.

6.5. Effects of photochemical transformation on the competition for N between phytoplankton and bacterioplankton

The photochemical reactions can convert biologically recalcitrant DON into bioavailable forms such as NH_4^+ (e.g. Moran & Zepp 1997, Vähätalo 2009) that can potentially stimulate the production of N-limited phytoplankton prevailing in the GoF for most of the summer (e.g. Kivi et al. 1993, Lignell et al. 2003). In the GoF, photoammonification occurs at rates (53–60 μmol NH_4^+ m^{-2} d^{-1}), comparable to those of atmospheric deposition (Vähätalo & Zepp 2005, Stedmon et al. 2007). However, the growth response of the autotrophs to the photochemical transformation of refractory DOM differed between the two studies conducted in summer 2005 in the W GoF. In June 2005, the labile photoproduced N clearly stimulated primary production (Vähätalo et al. 2011), agreeing with the results of previous experiments conducted in our study area, in which photochemically released N led to positive phytoplankton biomass responses (Vähätalo & Järvinen 2007).

In contrast, the production and biomass of the initially N-limited phytoplankton in late summer 2005 were not enhanced by sunlight exposure of DOM (IV), probably resulting from intensified bacterial competition for N via relaxation of bacterial C limitation by photochemical release of LDOC. Sunlight exposure of terrigenous DOM may significantly increase the nutrient demand of estuarine bacteria (Smith & Benner 2005), and photochemical release of LDOC also significantly enhanced bacterial production in our study (I). Agreeing with this view of intensified bacterial nutrient competition, a recent mesocosm study of nutrient-organic C interactions in an Arctic pelagic ecosystem showed that LDOC addition to the plankton system with C-limited bacteria led to reduced phytoplankton biomass and activity (Thingstad et al. 2008). Similarly, the addition of labile C negatively affected the growth of picocyanobacteria in an experimental setup with the < 2-μm freshwater plankton community (Drakare 2002).

The contrasting responses of the production and biomass of phytoplankton to labile photoproducts in the experiments

conducted in June (Vähätalo et al. 2011) and in mid-July–August (IV) could have resulted from differences in the composition of the DOM pool, nutrient availability, grazing or changes in species composition. The temperature increase from 10 °C in June (Vähätalo et al. 2011) to 18 °C in mid-July markedly enhanced bacterial growth (Autio 1998), since the bacterial growth rate (biomass-related production) in the sunlight-exposed samples was on average three-fold higher than in June (Vähätalo et al. 2011). Low temperatures more affect the growth of heterotrophic compartments of the plankton systems, including bacteria and protozoa, than algal growth (Pomeroy & Deibel 1986, Rose & Caron 2007). It thus appears that in our study area the positive effect of photoproduced labile N on the growth of phytoplankton (Vähätalo & Järvinen 2007, Vähätalo et al. 2011) could be more pronounced in early summer, due to temperature limitation of bacterial growth.

6.6. Composition of the summer bacterial community and its responses to photochemical DOM transformation

The bacterial community in the surface water of the Baltic Sea is uniquely adapted to brackish water conditions, containing both freshwater and marine phylotypes (Riemann et al. 2008, Herlemann et al. 2011). The clone library showed that a large part of the bacterial community in the GoF surface water in July 2005, consisted of Actinobacteria, α-, β- and γ-Proteobacteria, Bacteroidetes and Cyanobacteria. At this broad phylogenetic level, the community composition was similar to those in previous detailed bacterial inventories from the central and throughout the Baltic Sea, acquired with pyrosequencing (Andersson et al. 2010, Herlemann et al. 2011). It contained typical freshwater groups, such as Actinobacteria (Jezbera et al. 2009) and β-Proteobacteria that often predominate in fresh waters and are also found in coastal areas in lower numbers (e.g. Bouvier & del Giorgio 2002). Typical marine groups were also abundant, including the SAR11 cluster of α-Proteobacteria, which appears abundant throughout the Baltic Sea, and SAR86 of γ-Proteobacteria, which increases with salinity in the Baltic Sea (Herlemann et al. 2011). Previous bacterial community data from GoF surface water in May, acquired with pyrosequencing (Koskinen et al. 2011), was in turn distinct from our late summer community, probably reflecting at least in part strong seasonal variation in bacterial community composition in the Baltic Sea (Andersson et al. 2010). In contrast to previous results from the Baltic Sea, we found no clones affiliated with Verrucomicrobia that are potentially well adapted to brackish conditions in the Baltic Sea (Riemann et al. 2008, Herlemann et al 2011).

Photochemical transformation of the DOM led to notable changes in the composition of the bacterial community, agreeing with previous experiments in fresh and marine waters (Judd et al. 2007, Perez & Sommaruga 2007, Abboudi et al. 2008, Piccini et al. 2009). The three bacterial OTUs that most benefited from the photochemical degradation of DOM belonged to β-Proteobacteria. Members of the β-Proteobacteria may respond positively to allochthonous organic C (Burkert et al. 2003), which was probably also the case in the present study, since photoproduction of labile DOM in the GoF is mainly attributed to transformation of allochthonous humic DOM (Stedmon et al. 2007, Vähätalo et al. 2011).

The results from this study support those from a coastal lagoon, where photochemical alteration of DOM increased the percentages of α- and β-Proteobacteria (Piccini et al.

2009). On the other hand, photochemical transformation of DOM from lake water, phytoplankton and adjacent soil decreased the percentage of *β*-Proteobacteria and increased that of the Actinobacteria of an alpine lake bacterial community (Perez & Sommaruga 2007). The overall effect of the photochemical alteration of DOM on bacterial growth was negative in the alpine lake study, and the varying composition of the original DOM pools probably contributed to the contrasting responses between the studies.

Few bacterial OTUs among the diverse bacterial communities appeared to clearly benefit from the photoproducts of biologically recalcitrant but photochemically reactive humic DOM. These bacteria, clustering within the order Burkholderiales, clearly utilize the labile DOM photoproducts effectively gaining competitive advantage over the other bacterial strains.

All three bacterial OTUs that benefited from the photochemical transformation of DOM were affiliated with genera that are typically encountered in fresh waters and have previously been associated with utilization of potential photoproducts. One OTU was affiliated with the genus *Polynucleobacter*, which is abundant in humic waters, including a lake in southern Finland (Burkert et al. 2003, Grossart et al. 2008, Taipale et al. 2009). A *Polynucleobacter* strain from Lake Kasumigaura (Japan) grew well in irradiated lake water, had a strict requirement for organic acids and showed highest growth rates in pyruvate, acetate and formate, which are common photochemical breakdown products (Watanabe et al. 2009).

The two other OTUs that benefited from the labile photoproducts were affiliated with the family Comamonadaceae, one with the genus *Hydrogenophaga* and the other with the genus *Acidovorax*. Both these genera are among the bacterial genera predominant in the assimilation of amino acids in lake snow aggregates (Schweitzer et al. 2001). Amino acids can be photochemically released from humic substances in fresh waters and coastal systems (Jørgensen et al. 1998, Bushaw-Newton & Moran 1999). In a freshwater ditch, members of *Acidovorax* were among the strains predominant in the degradation of malonates (Kniemeyer et al. 1999), which in turn have been identified as biologically labile photoproducts in natural waters (Backlund 1992).

The OTU affiliating with *Hydrogenophaga* also reacted positively with a rapid increase in the biomass of filamentous cyanobacteria at the end of the experiment, showing that labile photoproducts may support the growth of species that react rapidly to DOM from different sources. The example of *Polynucleobacter*, with a strict requirement for low-molecular-weight organic acids, indicates that the bacterial strains benefiting from labile photoproducts include specialists having a high affinity for photoproducts, such as low-molecular-weight carboxylic acids (Watanabe et al. 2009, this study). Since the photoproducts contribute from 2 % to 9 % and from 10 % to 11 % to the bacterial assimilation of DOC in coastal and fresh waters, respectively (I, II, IV; Vähätalo 2009, 2011), an adaptation to utilize them is expected. These results suggest that adaptation to utilize labile photoproducts may partially explain the high abundance, e.g. of *Polynucleobacter* in humic waters (Burkert et al. 2003, Grossart et al. 2008, Taipale et al. 2009). The abundance of such specialists is probably greatest during summer in humic lakes with the highest concentrations of DOM photoproducts, but they may also play important roles in the utilization of photoproducts from terrestrial DOM in coastal waters.

The finding of bacterial groups that are adapted to utilizing specific components of the DOM pool implies that the abundance and activity of such indicator species (e.g. *Polynucleobacter*) could be used to approximate the importance of these DOM components (and possibly DOM sources) for bacterial nutrition in the Baltic Sea. Such an approach would be, to some extent, masked by the effects of host-specific viral lysis on the community composition. Nevertheless, it could provide useful information on this subject that would be difficult to achieve with other approaches.

7. CONCLUSIONS

During the season of phytoplankton growth, autochthonous DOM accumulated in the surface layer of the GoF. The dynamics of the DOM accumulated in the GoF are summarized with a conceptual model of its net flows (Fig. 5). The DOM pool functioned as a notable temporary storage for C fixed by phytoplankton, since the total DOC accumulation during the stratified period was about 20-fold higher than the average phytoplankton biomass and about 30 % of the contemporary autochthonous production in the surface layer during the same period. The accumulation of DOM enabled its temporary export out of the surface layer, e.g. by turbulent mixing and downwelling caused by strong winds during the stratified period and by convection and mixing of the entire water column during the autumn overturn. The export of DOC out of the surface layer was estimated to contribute 12–25 % of the reported vertical POC export in the GoF, thus facilitating the drawdown of atmospheric CO_2 during the productive season. The extent to which the DOC exported is degraded in the Baltic Sea and released back into the atmosphere as CO_2 is another question deserving further investigation.

The bacteria were primarily C-limited, and accumulated LDOC was hence assumed to be degraded in the surface water and lost mostly as CO_2 via respiration in the microbial loop during the stratified period. The concentrations of LDOC were low in GoF surface water during most of the productive season, suggesting that most of the easily degradable DOC released from the plankton food web was effectively consumed by bacteria, while the accumulating DOM pool consisted mostly of semilabile or refractory compounds. In the GoF, the efficiency of the plankton system in incorporating anthropogenic CO_2 emissions is thus lower than a situation in which nutrient limitation of bacteria would allow accumulation of LDOC in the surface layer and subsequent export of LDOC to deep water (cf. Rivkin & Anderson 1997, Thingstad et al. 1997).

Another important consequence of bacterial C limitation is that it restricts bacterial nutrient consumption, directing the nutrient flow from a "microbial link" towards a "classical" large algae-mesozooplankton food chain, because small algae are again strictly grazing-controlled (Thingstad et al. 2007, 2008). The slower predator-prey responses in the "classical" food chain than in the microbial (bacteria-small algae-ciliates) food web may increase the duration of algal blooms (Thingstad et al. 2007, 2008), and hence C limitation of the bacterial assemblage may eventually enhance the blooms of filamentous cyanobacteria in the northern Baltic Sea.

In late summer, the actively growing and decaying bloom of N_2-fixing cyanobacteria released extra LDOM into the surface water, allowing accumulation of LDOC and LDON. Bacterial production in deep water of the GoF was C-limited, implying that the LDOC

accumulated in the surface water represented a potentially important substrate supply for deep-water bacteria during vertical transport events.

During the stratified period, the LDON concentration averaged 11 % of the total DON pool, demonstrating that total nutrient concentrations and ratios should not be used in determination of which nutrient (N or P) limits the plankton system in the GoF. Nevertheless, the LDON concentrations that ranged from 0 to 4 μmol C l^{-1} formed up to 95 % of the available N pool (DIN and LDON) during the productive season. Thus, the variability in the LDON pools is of major importance for the nutrition of the N-limited plankton community in the GoF.

Photochemical transformation of DOM concerns mainly humic DOM of terrestrial origin in the GoF. The photoproduction of LDOM supported < 10 % of the bacterial production in the mixed surface layer of the GoF during summer, thus forming a relatively small link between terrestrial DOM and planktonic production in the open-sea water. Nevertheless, photoproduced LDOM notably affected the composition of the bacterial community and part of the bacterial community appeared to be specifically adapted to utilizing photoproducts. Labile photoproducts mainly supported the growth of typical freshwater species. Since labile photoproducts are released mainly from DOM of terrigenous origin, the photolysis of humic DOM that enters the Baltic Sea with freshwater inflows provides a natural explanation for the identity of the photoproduct-utilizing bacteria. It appears that the successful immigration of freshwater bacteria to surface waters of the Baltic Sea could be affected by adaptations to utilizing labile photoproducts.

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